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ORIGINAL ARTICLES

HAY FEVER AND CERTAIN OTHER LOCAL ANAPHYLACTIC PHENOMENA REFERABLE TO THE RESPIRATORY MUCOUS MEMBRANES*

BY A. PARKER HITCHENS, M.D., AND CLAUDE P. BROWN, M.D., GLENOLDEN, PA.

IN reviewing the history of hay fever, we find that the literature naturally falls into five fairly distinct periods:

First Period.—In the earlier bibliography, long before the first accurate description of the disease by Bostock[1] there are numerous references to periodical attacks of rhinitis and asthma as well as to various idiosyncrasies associated with flowering plants. Among the earliest of these is Botallus[2] (1565) "for there are many who are attacked with sneezing, by the slightest thing whatsoever, others by merely smelling a rose."† Others mentioned by Sticker[3] are Binninger[4] (1673), Ledelius[5] (1684), Hünerwolf[6] (1687), and Constant de Rebecque[7] (1691). Bostock stated that the earliest reference to which his attention had been called was that of Herberden[8] (1802). The quotation referred to is probably, "Five patients were attacked violently by this disease for a month every summer; one was afflicted annually for the entire summer; another was never free from it except in the summer."‡ These references are sufficient evidence that hay fever existed for centuries before its recognition as a specific disease, and all attempts to estimate its antiquity are entirely futile.

Second Period.—In 1819, Bostock[1] described a "Case of a Periodical Affection of the Eyes and Chest," so accurately that later writers, even in their

*Presented at the Second Pan-American Scientific Congress, Washington, D. C., Sec. viii, Jan. 5, 1916.

†"Nam plerique sunt qui quacunque re levissima sternutationis multis agitabuntur, alii ex solo rosae odoratu."

‡"Quinque aegris contigit graviter laborare hoc morbo per mensem omni aestate; alium totam aestatem afflixit quotannis; alius nunquam nisi aestate ab eo liber fuit."

In a translation of Heberden's work published the next year[9] is the following: "I have known it return in four or five persons annually in the months of April, May, June, or July, and last a month with great violence. In one a catarrh constantly visited him every summer; and in another this was the only part of the year in which it ceased to be troublesome."

most elaborate analyses of symptoms, have been unable to add anything of clinical importance. The attention of physicians thus being called to this affection, reports in a short time began to appear in the medical publications of England, France and Germany. Nine years later Bostock[10] himself added distinct accounts of 18 cases with 10 others "less correctly ascertained." Elliottson[13] contributed a number of cases and noted many interesting data with regard to the association of symptoms with the apparent exciting causes. From the contributions of Gordon[11] and Macculloch[12] we learn that the term "hay fever" was in common use some time before its recognition as a disease by Bostock.

Our information concerning the incidence of hay fever, its relation to age, sex, and condition of life, and its geographical distribution may be attributed in some degree to Elliottson,[13] but still more to Phoebus.[14]

With regard to the etiology, various theories were held and practically every external agency was claimed at first as an exciting cause. Bostock believed his own symptoms to be due to the heat of summer, while other writers held the odors of flowering plants chiefly responsible. The proposal of a bacterial origin by Helmholz[15] never gained much headway. Pirrie[16] suggested that the disease was of nervous origin, at the same time admitting emanations from plants and various external agencies as exciting causes.

In 1859, Professor Phoebus[14] of the University of Giessen sent out about 400 circulars to physicians, medical societies, etc., requesting information concerning hay fever. His analysis of the replies received remains one of the best statistical studies of the disease, but adds no fundamental fact with regard to etiology. One of the most interesting communications quoted by Phoebus[14] was that of Kirkman who disagreed with the opinion that emanations from Anthoxanthum odoratum were a cause (probably *the* cause according to Gordon[11]), because "I am always attacked at least three weeks before the Anthoxanthum is in blossom." Later in his hothouse he noticed, a day or two before Christmas, a single Anthoxanthum odoratum in blossom and well loaded with pollen. He rubbed the flower in his hand and sniffed it, whereupon all the symptoms of hay fever appeared immediately, continued for an hour and then left him.

In 1872 and 1876, respectively, Morrell Wyman[17] and Beard[18] published statistical investigations similar to those made by Phoebus. Although Blackley's work had appeared three years earlier, Beard was confident that his theory of a nervous diathesis was conclusive; he said that pollen was merely one of the "debilitating influences, exciting causes purely—and of themselves are powerless to induce, or at least to sustain an attack."

Third Period.—The chaotic state of these earlier opinions could be cleared up only by actual experiment. Without attracting much serious attention at the time, this necessary elucidation was furnished by Charles H. Blackley,[19, 20] a physician of Manchester, England. In 1873, he published the results of one of the most complete researches in the history of experimental medicine. Blackley, himself a sufferer from hay fever, was at first inclined to agree with Bostock that summer heat was the cause of the disease, but he was led to question the correctness of this view by circumstances connected with a journey to the seacoast. In the locality of his home at Manchester the hay had already been

gathered in and his attacks of hay fever had ceased; on approaching the seacoast, however, the symptoms reappeared, and later investigation revealed a field of uncut hay much of which was in flower. His attention was directed to the same subject by an incident two years later. Upon examining a bunch of grass brought indoors by one of his children, the cloud of pollen scattering near his face brought on violent sneezing in two or three minutes. Blackley dates his experimental work from this time (1859).

After carefully reviewing all the current opinions concerning the etiology of hay fever, Blackley asked himself the following questions:

1. Can pollen produce the symptoms of hay fever?

2. Does this property belong to all the pollens, or is it confined to the pollen of some one or more orders of plants? And if so, to what natural orders does it belong?

3. To the pollen of which natural order, or of which species of this order, are the actual attacks of hay fever, as they occur in early summer, due?

4. Is this condition or property found in the dried as well as in the fresh pollen?

5. To what special substance in pollen is this supposed action due?

In order to answer these questions Blackley experimented upon the pollen in five different ways: "(1) by applying it to the mucous membrane of the nares; (2) by inhaling it, and thus bringing it into contact with the mucous membrane of the larynx, trachea, and bronchial tubes; (3) by applying a decoction of the pollen to the conjunctiva; (4) by applying the fresh pollen to the tongue, lips, and fauces; (5) by inoculating the upper and lower limbs with the fresh moistened pollen."

Blackley[19] tested on himself the pollens of the grasses and of plants belonging to 35 other natural orders, all of which produced symptoms. He studied microscopically the various pollen grains; furthermore, he counted the grains deposited within a given time upon slides coated with glycerine and carbolic acid. These studies were made at different seasons, indoors and out, in country and in city, at about the level of a man's head and at higher altitudes by means of kites. Blackley demonstrated that symptoms did not appear until the pollen grains had reached a certain number, and that the severity of the symptoms varied in direct ratio with the amount of pollen in the air. He also showed that pollen may travel enormous distances, thus accounting for the fact that the seashore, or even the ocean, does not always afford relief to susceptible persons.

In all this work Blackley himself was the subject of the experiment. He tried in vain to find other hay fever sufferers who would submit to the tests.

In spite of the evidence presented by Blackley, the attention of his contemporaries was so concentrated upon the work of Pasteur and Koch that no suggestion regarding etiology was welcomed unless founded upon bacterial infection. The findings of Helmholz that a vibrio was the cause of hay fever were confirmed by Binz[23] and Patton.[24] Heymann and Matzuschita,[22] however, attempted to straddle the question by suggesting pollen as the carrier of bacteria, especially of streptococci.

Furthermore, the fact that Blackley's work contained no facts of direct therapeutic value naturally detracted from its significance for clinicians. Thus it was not until the successful application of Noon's method of vaccination that the etiologic role of pollen attracted general interest and found final acceptance to the exclusion of all other theories.

Fourth Period.—In 1903, Dunbar[21] published his work built upon the foundation laid by Blackley. With technic similar to that used by his predecessor, Dunbar experimented upon himself and other susceptible persons with the result that pollen was established as the cause of hay fever—thus the findings of Blackley were confirmed. Dunbar believed at first that only certain pollens were in question. It is probable, however, that the pollen of any plant may be a cause of hay fever; although those varieties found in largest quantities in the atmosphere undoubtedly claim the greatest number of sufferers.

Dunbar was more fortunate than Blackley in having at his disposal the results of all the later achievements in both organic chemistry and immunology —the latter was an unknown science in Blackley's day. Dunbar's work on the chemistry of pollen thus constitutes a real advance in our knowledge of hay fever. In the field of immunology he was able by serological tests to strengthen the evidence in favor of the etiologic role of the pollen proteins. He was the first to establish the possibility of active immunity and to elaborate a specific therapy, but he fell into error by applying to hay fever the discoveries of Behring, Kitasato and others concerning bacterial toxins and antitoxins. Dunbar maintained that hay fever was caused by a true toxin existent in the pollen and that the serum of animals (rabbits and horses) injected with pollen and pollen derivatives possessed true antitoxic power. At present we regard hay fever as an anaphylactic phenomena and consider that the serum elaborated and patented by Dunbar* belongs with the antibacterial serums; in other words, that it depends for its activity upon amboceptor and not upon antitoxin.

Fifth Period.—In 1911 Noon[25] reported the results of his work in the laboratory of Sir Almroth Wright on the treatment of hay fever with subcutaneous injections of pollen extracts in minute doses. Curtis[26] and Dunbar had previously used plant extracts and pollen extracts, but neither had achieved results sufficiently promising or reliable to encourage a continuation of this work. Noon injected extracts of timothy pollen controlling the doses by ophthalmo reactions; this work interrupted by his untimely death was continued by Freeman.[27]

Preparation of Pollen Extracts.—Dunbar[28] and his associates Prausnitz,[29] Kammann,[30] Weichardt[31] and Liefmann[32] demonstrated that the protein which constitutes about 40 per cent of the organic substances in pollen is the active agent in causing hay fever. Accordingly all methods in the preparation of material for vaccination must include extraction of this essential protein.

One of the methods used by Dunbar for the preparation of his "Pollen Toxin" is to extract the finely pulverized pollen with 5 per cent sodium chloride solution containing 0.5 per cent phenol, incubating the mixture at 37° C. The undissolved portion removed by centrifugation consists of empty pollen membranes and inactive starch rods. The supernatant opalescent solution contains

*D. R. P., No. 152163.

the dissolved proteins: this is intensely active, even in high dilution, when applied to the skin or conjunctiva of susceptible persons. For use, the extract is diluted with physiological saline solution. Dunbar suggests further purification by precipitation with eight (8) volumes of absolute alcohol or by dialysis. Only the albumens, which constitute about 16 per cent of the total proteins, are toxic, the globulin fraction being entirely inactive.

Subsequent investigators have made their extractions with distilled water and with saline solutions of various strengths. The pollen is sometimes ground with sand; Goodale considers grinding unnecessary.

There are other modifications in technic which are scarcely worth mentioning. In many instances the possibility that the extract is not sterile cannot be eliminated. Standardization is generally considered accomplished by noting the relation between the quantity of fluid and the amount of pollen extracted. This procedure is obviously subject to great variation. Cooke's method of standardization seems to be the most accurate so far and it is the one we have adopted.

Recent Literature.—Among the recent reports of Clowes,[35] Lovell,[36] Lowdermilk,[37] Ulrich,[38] Koessler,[39] Manning,[40] Cooke,[41] Wood,[42] and Goodale,[43] the observations of Ulrich regarding intervals between doses are of special interest. He noted that some patients were relieved for much longer periods than others and because of this variability in individuals, he suggested that the intervals be governed in each case by the date of return of the symptoms.

The excellent technic of Cooke with regard to standardization has already been mentioned. His theories regarding the mechanism of hay fever and its treatment are in accord with the latest views on anaphylaxis.

"1. *With regard to antibody formation.*—It must be borne in mind that any form of foreign protein parenterally introduced within the living body, gives rise to the formation of a specific immune or antibody which exists either attached to certain cells or free. When union takes place between protein and free antibody, there is no clinical evidence of a reaction, but when a union takes place between protein and fixed antibody, a reaction takes place, and the nature of this reaction is determined by the type of cell to which the antibody was attached.

"2. *Relation between immunity and anaphylaxis.*—They are qualitatively identical but quantitatively different. In other words, when there is a large excess of antibody circulating free, we have an immune state; and when there is little antibody, and that for the most part attached, we have the sensitized state.

"3. *Duration of immunity.*—Immunity in the sense that free excess antibody once present is always present, does not exist. With cessation of protein injection, the body returns to the anaphylactic state, in which it may remain, or to the state of accelerated capacity to form antibody."

The attempts of Goodale to classify the susceptibility of patients according to natural orders and thus to study this part of the problem systematically, opens up many interesting possibilities not only in hay fever treatment but also in botanical classification.

Work of the Writers.—For obtaining the pollen the flowers are gathered just when pollination has started. The flowers are dried and the pollen collected by means of fine sieves. The pollen itself is thoroughly dried immediately and preserved in the dry state until it is to be extracted.

Preparation of Extract.

1. The pollen is mixed with sufficient physiological saline solution (0.85 per cent) to make a fairly thick paste.

2. The paste is transferred to a porcelain ball mill and ground for 24 hours, or, until microscopic examination shows that the pollen grains are broken.

3. Physiological saline solution is added and the resultant mixture is centrifuged to remove insoluble debris.

4. The extracted protein is purified by precipitation with acetone.

5. The precipitate is dried and thus preserved until needed.

6. For use, the precipitate is dissolved in physiological saline solution. The amount of protein-nitrogen in this solution is determined by the Kjeldahl method.

7. The solution is then diluted so that each cubic centimeter will contain certain fractions of a milligram of protein-nitrogen. The lowest dilution, 1 c.c. of which may be used as the initial dose in treatment, contains 0.0025 mg.

8. The final solutions are preserved from contamination by the addition of 0.25 per cent tricresol and sterilized by filtration. Sterility is determined by careful aerobic and anaerobic cultural tests.

Plan of Treatment.

Beginning with the minimal initial dose (0.0025 mg.) the treatment may be continued with increasing multiples of this amount according to the needs and the sensitiveness of the patient. The injections are first given at about 5-day intervals, but as soon as the period of relief has been found these intervals are shortened or lengthened, that is, if treatment is necessary during the season.

We consider this better technic than to depend upon ophthalmo reactions which may be dangerous or upon skin tests which merely complicate the procedure for the clinician. In other words, ophthalmo reactions and skin tests bear the same relation to pollen vaccine dosage that the opsonic index bears to bacterial vaccine dosage. By the ophthalmic test Noon and Freeman were able to place pollen vaccination upon a scientific basis similar to that achieved by Wright by means of the opsonic index technic. At present by starting with a dose (of either vaccine) demonstrated to be sufficiently small to do no harm, satisfactory results are obtained without the same control of dosage that was necessary at the beginning.

Our spring extracts contain a mixture of the pollens of red top timothy, rye and orchard grass; the autumn type consists only of ragweed.

These vaccines have been used by ourselves and by other physicians kindly cooperating with us in the treatment of 62 patients.

Of these 62 cases

18 had asthma as a complicating symptom.

Of these:

3.....no report.
1.....not relieved.
3.....considerably relieved.
11.....entirely relieved.

44 remaining cases.

Of these:

3.....no report.
2.....not relieved.
4.....slightly relieved.
18.....considerably relieved.
17.....entirely relieved.

One patient has apparently been cured; he was treated during two years in both spring and autumn. Another patient who was only partially relieved, was found to be susceptible to wheat, but he was compelled to leave the vicinity before a special extract could be prepared for him.

One patient not completely relieved by the "spring" vaccine was found susceptible to daisy pollen. A special "daisy" vaccine was prepared and the administration of a single dose brought complete relief.

Our results on the whole do not differ greatly from those obtained by others since Noon's first report. In none of these cases have we considered the possibility of a superadded or even possibly of a primary bacterial infection. This point, however, should not be overlooked.

Alexander reports two patients suffering from chronic nasal catarrh which condition was aggravated by pollen during the hay fever season. Treatment with Noon's Pollen Vaccine had no effect. But after bacteriological examination and the administration of Micrococcus catarrhalis vaccine, the cases were cured.

We have treated one asthmatic patient with bacterial vaccine, who throughout one winter did not have a single attack, although each winter previously she had experienced many.

Other Anaphylactic Phenomena.—The validity of the so-called idiosyncrasies to the emanations from animals cannot be questioned. Inquiry into the cases of sudden death following the injection of horse serum has shown that many of these persons had previously suffered asthmatic attacks whenever coming in contact with horses.

The manifestations of anaphylaxis after eating certain foods are now well recognized. All these facts lead to the deduction that any protein in the atmosphere may find hypersusceptible individuals who react with sneezing and all the symptoms of hay fever or asthma, or possibly with other more obscure symptoms. A case in point is the effect of bad ventilation (or the lack of ventilation) on certain persons while the other occupants of the room remain unaffected. Rosenau and Amoss[33] have attempted to demonstrate the presence of proteins in the exhaled breath, and although this work as yet lacks confirmation we believe it is a line of research worthy of closer attention.

Discussion.

Hay fever at present is a problem of immunology and of chemistry; and the extension of our knowledge is limited to these two fields unless some unsuspected development may lead to a departure from the present trend of research —a departure such as followed the investigation of anaphylaxis by Rosenau and Anderson.

The demonstration made by Noon that injection of a pollen extract, under the rules laid down by Wright, has immunizing and therapeutic value in hay fever, suggests two important questions:

1. *What essential constituent of the pollen should be contained in the extract?*—This is practically Question No. 5 asked by Blackley, and the work of Dunbar and his associates has gone far toward giving us an answer. However, the lack of a standard method for preparing an extract of maximum diagnostic, immunizing and therapeutic value, clearly indicates the intensive investigation needed in this direction; whether or not the same constituent would possess all these properties would be one point demanding further study.

In certain respects, the method of preparation adopted by us yields a product superior to those prepared by the technic described in recent reports.

a. *Keeping qualities.* Koessler[39] among others found his extracts to be worthless after 3 weeks. An extract prepared by the technic given above yielded positive therapeutic results after 2 years; chemical tests according to the Kjeldahl, biuret and Sörenson methods showed no change in protein-nitrogen content nor decomposition with formation of peptones or amino acids.

b. *Uniformity.* The Cooke Method of Standardization certainly appears more rational than that used by Dunbar and others who estimate the strength of the extract by the amount of pollen submitted to extraction. Lack of uniformity in the strength of his vaccines may account for the fear of anaphylactic shock expressed by Lowdermilk.[37] None of our patients had more than a slight local reaction which caused no inconvenience.

2. *To what pollens is the individual patient susceptible?*—This is Blackley's Question No. 2. While certain patients are susceptible to the pollen of only one or a very few plants (or at least to a single natural order), others, like Blackley, are susceptible to nearly all plants; there are, of course, all grades between. Goodale has attacked this problem from a practical standpoint by classifying the various pollens with a view to minimizing the number requisite in treatment. Freeman reduced his vaccine to an extract of timothy pollen alone. It would greatly simplify matters for the clinician if in the fall the pollen of a single member of the Compositæ could be applied. A sufficient number of cases has been reported however to show that some patients would not be relieved, even assuming that timothy pollen extract and ragweed pollen extract are adequate for the Gramineæ and the Compositæ respectively. Persons susceptible to rose pollen or to the pollen of certain trees are cases in point.

Although these two questions are the most important at the present time, a third problem is ever present with the practicing physician.

3. *In the present state of our knowledge of hay fever, how can this knowledge be best applied to the advantage of our patients?*—The answer to this question may be considered in two sections; (a) Measures to be taken two or three months before the hay fever season, and (b) Measures to be taken immediately before or during the hay fever season.

a. *Measures to be taken two or three months before the hay fever season.* When the patient can be studied beforehand, a survey of his habitual surroundings should be made. After noting all the flowering plants which might reason-

ably come into question, skin tests should be made with pollens of each of these plants in order to determine which of them are responsible. In this connection it must be remembered that pollens may travel great distances (Blackley); accordingly, a field of grain even several miles away must be taken into account.

It seems scarcely necessary to mention the eliminations that could be made according to the seasonal incidence of the attack. The Gramineæ in the spring, the Compositæ in the fall would receive first consideration.

b. *Measures to be taken immediately before or during the attack.* If the attack has already started, treatment should be begun at once with a vaccine representing the pollens most likely to be responsible for the attack. If the treatment does not give entire relief, an exact diagnosis may be made quite independently of the treatment.

While the ordinary seasonal attacks can be controlled fairly easily, those patients who suffer from earliest spring until latest autumn may present greater difficulties. As stated above, we have treated one patient of this type during two springs and autumns with apparent cure as the result.

As regards dosage we believe that the placing of extracts upon the conjunctiva should be unqualifiedly condemned. Skin reactions may not be quite so exact, but they are adequate for all practical purposes, if indeed any such control is needed, except in diagnosis.

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CEREBELLAR LOCALIZATION IN THE LIGHT OF RECENT RESEARCH*

By Davidson Black, B.A., M.B., Cleveland, Ohio.

IN 1870 Hitzig and Fritsch localized certain motor centers in the cerebral cortex by means of electrical stimulation and thus furnished the necessary experimental proof for the theory of cortical localization previously formulated by both Broca and P. Gratiolet.

Since this date many attempts have been made to demonstrate an analogous localization of function in the cortex of the cerebellum. However, indubitable proof of the underlying truth of this concept has only recently been forthcoming.

The cerebellar cortex, unlike that of the cerebrum, presents the same microscopic structure throughout its entire extent. On this account, no facts can be adduced in support of a theory of cerebellar localization on purely histological grounds. It is to researches within the last decade in the fields of comparative morphology, experimental physiology and clinical observation that we are chiefly indebted for our present knowledge of cerebellar localization.

Data of Comparative Morphology.

One of the great obstacles in the path of cerebellar research has been the persistent use of a system of nomenclature for the parts of the human cerebellum which is entirely lacking in morphological significance. Another source of confusion lies in the attempt to use the highly specialized human cerebellum as a type with which to compare the simpler cerebella of lower mammals. It is chiefly due to the work of Elliot Smith and Bolk that these obstacles have been removed and a rational method of description introduced.

These authors independently investigated the phylogenetic and ontogenetic morphology of the mammalian cerebellum and were able to recognize a definite plan of cerebellar structure common to all mammals with the possible exception of Monotremes.

Both investigators agree that the mammalian cerebellum consists of two major subdivisions, an anterior lobe and a posterior lobe, separated by a fissure of great constancy termed the fissura prima. The classic description of a cerebellum which is composed of a median lobe or vermis and two hemispheres has been rejected as being incorrect.

Figs. 1 and 2 are reproductions of the schemata constructed by Elliot Smith and Bolk to illustrate the morphological arrangement of the parts of the mammalian cerebellum.

The further researches of Bolk have culminated in the formulation by him of a theory of cerebellar localization based upon the data of comparative anatomy.

The parts of the cerebellum are subject to great variation in their relative development in different mammals. For example, in one group (Ungulata) the postero-median lobule is very large while the lobuli ansiformes are small; in

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another group (Carnivora) the lobuli ansiformes tend to be greatly developed while the postero-median lobule is small. Equally great variations of form and size occur in the other cerebellar subdivisions and especially is this true of the formatio vermicularis which is rudimentary in the elephant and man but in the Cetacea forms the largest part of the lobus posterior. That this great variability cannot be accounted for on the basis of body bulk is evident when one compares the simple postero-median lobule in the mole and the elephant with the much folded and complex posterior median lobule in the cat and horse.

Since form is largely dependent upon function, Bolk sought to explain these

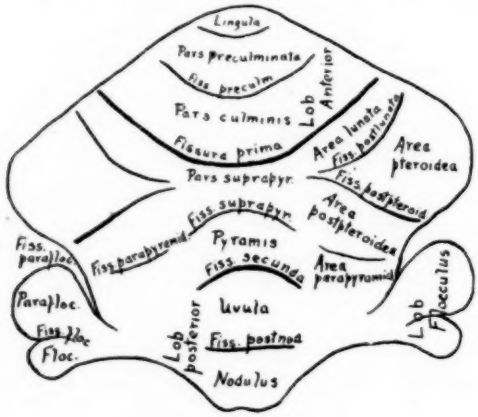

Fig. 1.

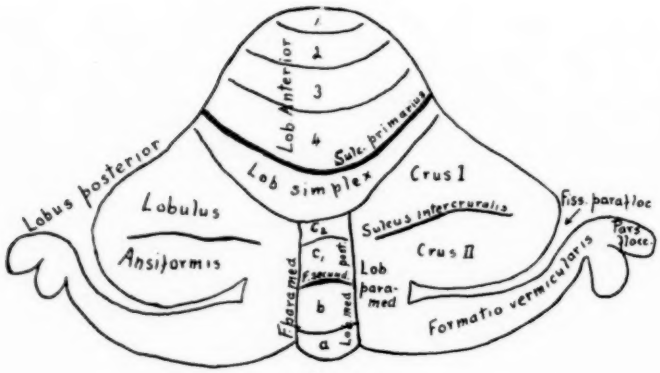

Fig. 2.

Figs. 1 and 2 represent schemata of the fundamental arrangement of the parts of the mammalian cerebellum spread out in one plane; Fig. 1, according to Elliot Smith (op. cit. 6) and Fig. 2 after Bolk (op. cit. 3 and 4). In their chief characters the two schemata resemble one another closely, such differences as appear being due in a large measure to the fact that Bolk has selected for his type the cerebellum of Lemur albifrons while Elliot Smith was influenced by his primary work on the cerebellum in the Edentata.

striking variations in cerebellar form by investigating the functional development of the muscular system, the direction of whose tonic, sthenic and static activities is the special function of the cerebellum.

Movements of certain regions of the body (for example the head, neck or trunk) necessarily involve the simultaneous action and coordination of the muscles of both right and left sides. On the other hand, the most complex move-

ments can be carried out by the musculature of one arm or leg while the homodynamic organs of the opposite side remain inactive.

If there be representation in the cerebellar cortex of centers concerned in the working of special muscle groups, it is reasonable to suppose that an unpaired center will prove sufficient for the coordination of muscle groups which are necessarily synergic in action. In the case of muscular areas capable of and accustomed to dysynergic action, a paired coordination center must be postulated. However, inasmuch as the limbs are frequently required to act in harmonious coordination one with the other (as for example in walking), an additional unpaired center becomes necessary in their case for synergic coordination.

Working upon the above hypothesis, Bolk successfully demonstrated a definite correspondence between the variations in the development of certain cerebellar lobules and the functional importance of certain muscle groups.

For further details, reference should be made to this author's monograph. The following is a brief synopsis of Bolk's deductions:

The cerebellar cortex is composed of a number of coordination centers some of which are paired and some unpaired.

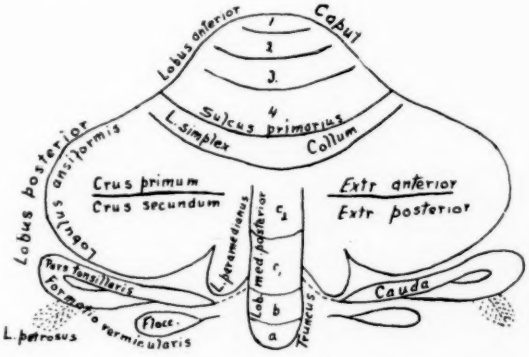

Fig. 3.—Schema of the parts of the mammalian cerebellum spread out in one plane. (After Bolk by Van Rijnberk from Luciani. Op. cit.) On the right side of the figure the relation of the different lobules to the functional development of the musculature is indicated according to the theory of Bolk noted in the text.

The *lobus anterior cerebelli* contains the coordination centers for the muscle groups of the head (eyes, tongue, muscles of mastication, muscles of expression) and also those of the larynx and pharynx.

The *lobulus simplex* contains the coordination centers for the muscles of the neck.

The upper part of the *lobulus medianus posterior* contains the unpaired centers (centers for synergic movement) for the left and right extremities.

In each of the *lobuli ansiformes et paramediani* are situated the paired centers for the extremities—the arrangement of the centers in each case being homolateral.

In the remainder of the cerebellum the coordination centers for the rest of the trunk and tail region are situated.

These facts have been further summarized and somewhat elaborated in the form of a diagram by Van Rijnberk which is here reproduced as Fig. 3.

For the purpose of correlating the current text descriptions of the human

cerebellum with the rational morphological subdivision of this organ according to Bolk, Figs. 4, 5 and 6 have been constructed. In view of the attached legend further description here is unnecessary.

Experimental Data.

With the establishment of the homologies of the cerebellar lobes among the various mammalian groups, it became possible to investigate their function experimentally in different forms. Van Rijnberk working in Luciani's laboratory, was the first to test Bolk's theory of localization by means of circumscribed extirpations of certain lobules. The results of his investigations confirmed Bolk's hypothesis and may be summarized in part as follows:

(1) Total or partial extirpation of the *lobulus simplex:* side to side oscillations of the head due to astasia of the muscles of the neck.

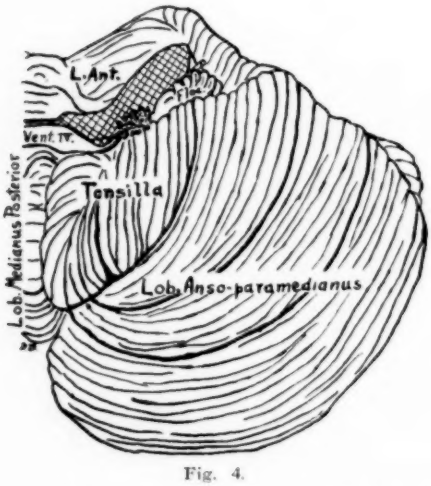

Fig. 4.

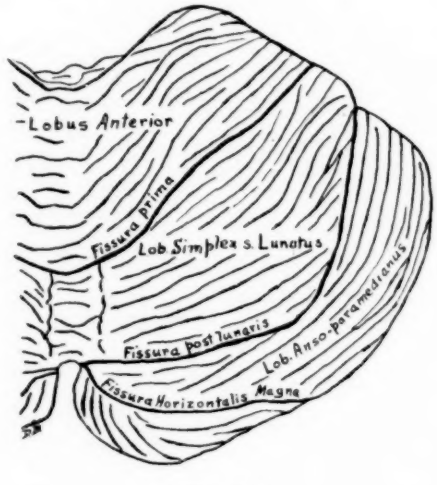

Fig. 5.

Figs. 4 and 5.—Diagrams to represent respectively a ventral view of the left half and a dorsal view of the right half of the human cerebellum illustrating the scheme of subdivision according to Bolk. (From photographs of specimens C. 193 and C. 194 from the Anatomical Museum, Western Reserve Medical School.) The Tonsilla and Flocculus are lobules of the Formatio vermicularis of Bolk.

(2) Complete extirpation of the *Crus primum:* homolateral dynamic disturbances in the fore-limb causing in the first irritative stages the assumption of a characteristic position, likened by the author to the military salute. Later occurred considerable dysmetria in the movements of the limb due to muscular atonia.

(3) Extirpation of the *Crus secundum:* homolateral asthenia of the muscles of the hind limb.

(4) Extirpation of *both crura of lobulus ansiformis:* marked asthenia and atonia in both fore and hind limbs on the same side as the lesion and the later appearance of a characteristic disturbance in walking termed the "hen's gait."

(5) Extirpation of the *lobulus paramedianus:* rotation on the longitudinal axis of the body associated with pleurothotonus to the operated side.

In each case the symptoms gradually became less marked and eventually almost completely disappeared, owing, according to Luciani, to organic compensation.

These observations of Van Rijnberk have since been confirmed in all their essentials by many investigators working on dogs, monkeys and other animals. In this connection the recent work of Rothmann and of André-Thomas et Durupt on dogs and monkeys should be noted.

These authors point out once more that the destruction of the cerebellar cortex does not give rise to a paralysis; as in man, cerebellar cortical lesions cause a perturbation in the equilibrium of antagonistic muscles: an anisosthenia. They have also carried the investigation of cerebellar localization a step further and have shown that, within the paired centers for the upper and lower extremities described above, there is a definite arrangement of subsidiary centers

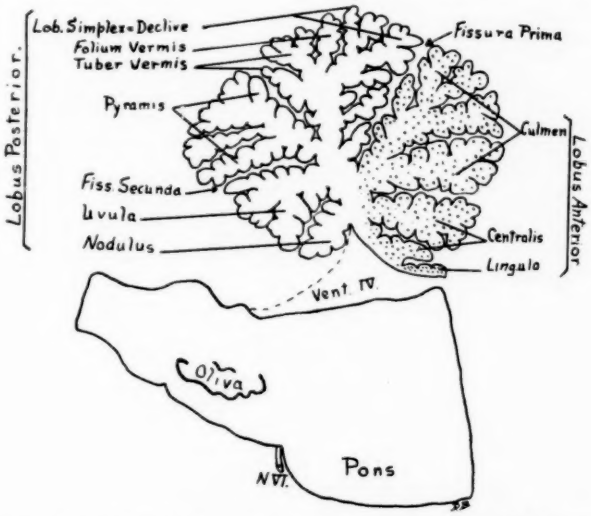

Fig. 6.—Diagram of mesial sagittal section through the human cerebellum to further illustrate the scheme of subdivision according to Bolk. (From specimen C. 192, Anatomical Museum Collection.) The old descriptive terminology for the parts of the so-called "vermis" is indicated for comparison. N. VI, Nervus abducens; Lobus anterior dotted.

for the direction of the activities of antagonistic muscle groups. Each of these secondary extremity centers is related to a single segment or articulation of the corresponding limb and presides over the elaboration of sthenic, tonic and static impulses distributed to definite muscle groups of the segment. As will be seen, these observations are in harmony with those of Barany based upon clinical investigations.

It is important to note that the symptoms of cerebellar deficiency after extirpation experiments, become gradually ameliorated and may, if the lesion be small, finally disappear. This is due to two influences: (1) possible organic compensation by the uninjured parts of the cerebellum itself which has already been mentioned, and (2) actual functional compensation by the purposive and voluntary acts of the cerebrum.

The influence of the cerebrum in the correction of the phenomena of cerebellar deficiency is of great importance. The destruction of one-half of the

cerebellum of a dog gives rise to such marked atonia, asthenia and astasia in the muscles of the same side, that the animal is at first unable either to maintain the erect position or to walk. The severity of these symptoms gradually lessens and after a time the animal learns to stand and to walk once more, though its position and gait are not entirely normal. If a second operation be now performed and the motor cortex of the contra-lateral cerebral hemisphere be destroyed, the symptoms of cerebellar deficiency return in their full severity and the power of standing in the erect position and of walking is permanently lost.

Clinical Data.

The diseases of the cerebellum which have furnished data of a useful nature in this connection are necessarily those caused by circumscribed lesions such as abscess, cyst or regional agenesia. With a positive diagnosis of cerebellar disease the question arises, is it possible to localize the site of the lesion with any degree of accuracy? In this connection reference may be made to the recent work of Rothmann, André-Thomas et Durupt, Babinski et Tournay, and especially to the theory of cortical localization within the human cerebellum, elaborated by Barany.*

On the basis of an extensive series of observations, Barany has described certain clinical tests by means of which he has been able to localize circumscribed cerebellar lesions with a considerable degree of exactitude. It will be of interest here to give a very brief resume of the procedure employed in carrying out these tests, on account of their diagnostic value.

Index Test.—("L'épreuve de l'index"—"Zeigeversuch.")

The patient's eyes being closed, he is asked to execute a simple movement in a given direction with one of his extremities. For example, the forearm being firmly supported, the patient's index finger is extended and brought into contact with that of the observer. The patient is then required to move his finger vertically downward and then to return it to its previous position. The test is repeated a number of times both in the vertical and in the horizontal direction and if any tendency toward deviation from the plane of movement be present, its direction is noted. By slight modifications of the foregoing procedures it is possible to test each of the limb segments in all positions of rotation, pronation or supination.

These tests may be practiced in two different ways: (1) without previously induced nystagmus, and (2) after the production of artificial nystagmus.

In the *normal subject* the following reactions may be observed:

(1) Without previously induced nystagmus, the tests in question are correctly performed—there is no deviation.

(2) After production of artificial nystagmus, a deviation may be noted in the direction corresponding to the slow jerk of the nystagmus. This is termed by Barany the "reaction deviation."

In the presence of a cerebellar lesion certain variations of the above reactions may be observed:

*It is of interest to record in the present connection that this author has but recently been awarded the Nobel Prize in recognition of his distinguished work in this field.

(1) When tested without previously induced nystagmus, a definite deviation of the tested segment may occasionally be noted. This is what Barany terms the "spontaneous deviation."

(2) After production of artificial nystagmus, an absence of the normal "reaction deviations" can be noted. It is this test which has proved to be most useful in diagnosis.

The perturbation or deviation which is observed during these tests, depending upon the direction in which it is produced and the segment of the limb in which it occurs, serves to indicate the exact site of the cerebellar lesion. This statement is based upon the fact that interruption of the cerebellar control of one muscle group leads to an exaggeration of antagonistic muscular action and thus to deviation of the segment during movement in the direction of the hyperactive muscle group.

Barany has thus been able to map out definite areas in the human cerebellar

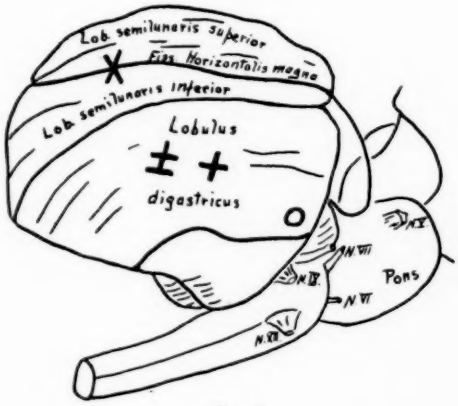

Fig. 7

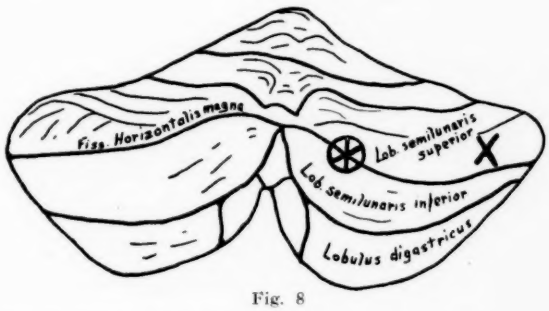

Fig. 8

Figs. 7 and 8 represent respectively the inferolateral and the posterior aspect of the human cerebellum indicating certain cerebellar localizations according to Barany. After Barany, from André-Thomas et Durupt. Op. cit.) See text for description. N. V, Nervus trigeminus; N. VI, Nervus abducens; N. VII, Nervus facialis; N. IX, Nervus Glossopharyngeus; N. XII, Nervus hypoglossus.

cortex, each associated with a special muscular action. The chief deductions this author has made from his observations may be summarized as follows:

(1) There exists an exact localization of function in the cortex of the human cerebellum.

(2) The centers for the extremities are situated in circumscribed areas in the cortex of the hemispheres.

(3) In correspondence with the theory of Bolk and with experimental researches, the centers for the right and left extremities are situated respectively in the right and left semilunar (superior and inferior) and digastric lobules.

(4) Within these chief centers the representation of the limb musculature is spatially determined by the action of its various functional groups and by their position in the limb. Thus, within the arm center, other subsidiary centers are to be recognized concerned in the movements of the limb in the horizontal plane, in the sagittal plane, in rotation, in pronation and in supination; and further, in

each subsidiary center the more minute arrangement of functional areas is in accordance with the movable segments of the limb.*

(5) On the sudden destruction of a center concerned in the movements of a limb in a determined direction (for example, to the right) there is produced a "spontaneous deviation" in the inverse direction (that is, to the left). This "spontaneous deviation" may disappear in time, either through the compensatory action of the cerebrum or of the cerebellum itself.

(6) The chief centers of direction which have been investigated are localized as follows: (vide Figs. 7 and 8).

⊗ A center for the tonus of the musculature concerned in the movements of the right arm downwards, is situated at the medial extremity of the right superior and inferior semilunar lobules. (Abwärtstonus Zentrum.)

× A center for the tonus of the musculature concerned in abduction of the right arm is situated at the lateral angle of the hemisphere in the right superior and inferior semilunar lobules. (Auswärtstonus Zentrum.)

O A center for the tonus of the musculature concerned in adduction of the right hand is situated in the anterior part of the right digastric lobule in the area immediately behind the labyrinth as it lies in the petrous bone.† (Einwärtstonus Zentrum.)

+ A center for the tonus of the musculature adducting the right arm is situated caudad and laterad from the preceding center, in the right digastric lobule.

± Still more laterad and caudad on the right side is situated a center for the tonus of the musculature adducting the right hip.

In the left hemisphere of course a similar arrangement of centers obtains.

It is evident from the foregoing account that the general theory of cerebellar localization as originally formulated by Bolk has been to a large extent confirmed not alone by experimental studies but also by careful clinical observation. Barany's localizations in the human cerebellar cortex remain yet to be confirmed in detail but the importance of his work in thus presenting a possible means of early diagnosis of cerebellar disease cannot be overestimated.

It will be of interest in conclusion to contrast the phenomena of motor localization characteristic of the cerebrum with those of the cerebellum and note the fundamental differences between cerebral and cerebellar control.

The cerebellar cortex has been shown to be practically inexcitable as compared with that of the cerebrum over the motor area.

Muscular representation in the cerebral motor area is chiefly determined by the segmental position of the respective muscles and broadly speaking, the more caudad muscles are represented in the upper portion of the motor area while the most cephalic groups are represented in the lowest areas of the precentral region. On the other hand in the cerebellum, while the grouping of the "tonus centers" has been determined in part by segmental position, their arrangement

*Barany has frequently demonstrated that "spontaneous deviation" (to the right or left in the sagittal plane) may exist in the hand while in the position of pronation but be entirely lacking when the position is changed to that of supination. Such clinical phenomena receive an explanation on the hypothesis of subsidiary centers.

†The subsidiary center for the tonus of the muscles adducting the hand when in the position of pronation, is more medially placed than the center for adduction of the hand when in the position of supination.

within the lobules has been chiefly determined by the functional association of muscular groups.

The cortex of the cerebellum is everywhere concerned in the elaboration of tonic, sthenic and static impulses of a reinforcing nature distributed for the most part homolaterally. A special part only of the cerebral cortex is concerned in the elaboration of impulses of a voluntary, motor, clonic nature distributed heterolaterally.

Destruction of the motor cortex on one side of the cerebrum gives rise to an actual paralysis of a spastic nature in the musculature of the opposite side of the body while destruction of the cortex on one side of the cerebellum causes no paralysis but gives rise to atonia, asthenia and astasia of the musculature on the same side of the body.

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L. Bolk: (1) "Hauptzüge der vergleichenden Anatomie des Cerebellum der Säugetiere, mit besonderer Berücksichtigung des menschlichen Kleinhirns"—Monstschr. f. Psychiat. u. Neurologie, Bd. 12, Heft 5, 1902, pp. 432-467. (2) "Beiträge zur Affen-Anatomie IV—Das Kleinhirn der Neuweltaffen"—Morphol. Jahrb. Bd. 31, 1902, pp. 44-84. (3) "Das Cerebellum der Säugetiere"—*Part I,* Petrus Camper, Vol. 3, 1905, pp. 1-136; *Part II,* ibid., pp. 455-598; *Part III,* ibid., Vol. 4, 1906, pp. 115-194. The theory of cerebellar localization is summarized and the problem of morphological variation in relation to function discussed in the third part of this communication, pp. 153-194. (4) "Das Cerebellum der Säugetiere"—Jena, 1906. This is a collection in book form of the researches published in Petrus Camper in three parts.

G. Van Rijnberk has published critical reviews of the current literature on cerebellar localization up to 1912—(1) "Die neueren Beitrage zur Anatomie und Physiologie des Kleinhirn der Säuger," Part III, Folia Neurobiologica, Bd. I. 1907, pp. 535-551. (2) "Weitere Beitrage zum Localisationsproblem in Kleinhirn." ibid. Bd. 6, 1912, pp. 143-170. Reference should also be made to *Luciani's* discussion of cerebellar localization in "Human Physiology," Vol. III. English translation from 4th Italian Ed., Macmillan & Co., 1915—pp. 473-485.

André-Thomas et Durupt, in their book "Localisations Cérébelleuses" (Paris, 1914), present the results of their researches upon dogs and monkeys together with an interesting and detailed review of the subject in all its phases and an extensive bibliography.

Babinski et Tournay in their communication "Le Symptomes des Maladies du Cervelet et leur Signification" read at the meeting of the 17th International Congress of Medicine in London have discussed the clinical aspect of this problem. An extensive clinical bibliography is also given in this report—Transactions, Section XI, Part I, 1912—pp. 51-58. The paper by *M. Rothmann* on "The Symptoms of Cerebellar Disease and Their Significance" (ibid., pp. 59-83) should also be consulted in this connection.

R. Barany has published a number of his important clinical observations under the title, "Lokalisation in der Rinde der Kleinhirnhemisphären des Menschen"—Wiener klinische Wochenschrift, No. 52, Dec. 26, 1912. His technic is described and a brief summary given in the papers by Babinski et Tournay and by André-Thomas et Durupt, cited above.

REGULAR ECTOPIC RHYTHMS*

By Frank N. Wilson, M.D., Ann Arbor, Mich.

IN the normal heart the sinoauricular node, a specialized structure which lies in the floor of the sulcus terminalis of the right auricle, has a higher degree of rhythmicity than any other portion of the cardiac tissue and it is in this node that the normal cardiac rhythm has its origin. Under abnormal conditions, however, some other part of the heart may initiate stimuli more rapidly than the sinus node or may be functionally separated from it by conduction changes and may thus become the cardiac pacemaker. Rhythms which arise outside of the sinus node have been called ectopic rhythms. Lewis[1] has long insisted that such rhythms should be divided into two classes, homogenetic ectopic rhythms and heterogenetic ectopic rhythms. In the first type the impulses are supposed to be generated by physiologic, in the later type by pathologic processes.

Regular Ectopic Rhythms of the Heterogenetic Type.

Heterogenetic rhythms are recognized by the following characteristics: (1) The impulses which give rise to them are formed at a very rapid rate. This may lie anywhere between 140 and 370 per minute. (2) The rate is remarkably constant and is little or not at all influenced by procedures which influence the normal cardiac rhythm such as change of posture, exercise, or stimulation of the vagus nerves. It is true that such rhythms sometimes cease abruptly upon the application of various therapeutic measures which involve vagus stimulation but the cessation of the abnormal rhythm is not usually preceded by any gradual slowing and the results of vagus stimulation are very inconstant. (3) There is a marked and abrupt change in heart rate at the onset and at the end of the abnormal rhythm. (4) The abnormal rhythm occurrs as a rule in paroxysms which cease abruptly and which are followed by a post-paroxysmal pause analogous to the pause which follows a premature systole. In fact such rhythms are believed to be made up of a series of premature beats arising at the same point. They probably arise only when the physiologic integrity of some small area of the heart muscle has been interfered with either by an organic lesion, by some toxic agent, or by nutritional changes.

Paroxysmal Tachycardia.

Clinically, we divide regular rhythms of the heterogenetic type into two classes, paroxysmal tachycardia and auricular flutter. Paroxysmal tachycardia usually arises in the auricular muscle, less commonly in the ventricular muscle or in the special muscular structures which unite auricles and ventricles. The heart rate during the paroxysm usually lies between 140 and 220 per minute. An example of an attack of paroxysmal tachycardia of auricular origin is shown in Fig. 1. It exhibits the typical features of a heterogenetic rhythm; the rapid rate (150 per minute), the abrupt and marked change in heart rate at its ter-

*From the Department of Internal Medicine, University of Michigan.

mination, and the post-paroxysmal pause. The ectopic nature of the rhythm is shown by the abnormal form of the P-wave of the electrocardiogram. This is inverted and occurs between the R- and P-waves of the ventricular complex. The last ventricular complex is not modified by one of these inverted P-waves, which indicates that the last ventricular contraction occurred in response to the auricular contraction which coincided with the previous ventricular systole. The prolongation of the P-R interval in combination with the rapid heart rate caused auricles and ventricles to contract simultaneously and thus produced the positive venous pulse seen in Fig. 1. This increase in the length of the P-R interval is common in paroxysmal tachycardia and is probably due to fatigue of the junctional tissues as a result of the rapid heart rate. A diagram of the cardiac mechanism is shown in Fig. 1, the heterogenetic character of the ectopic rhythm

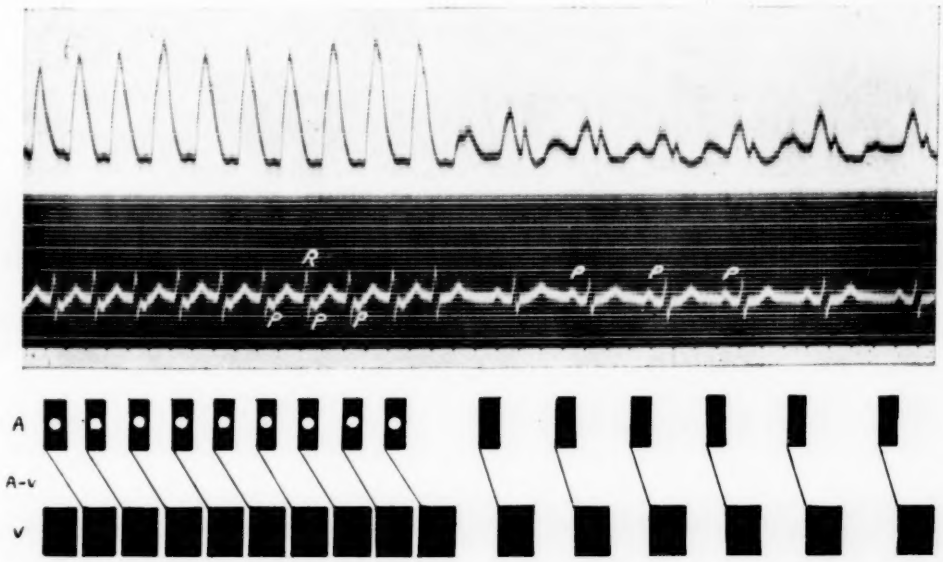

Fig. 1.—Lead II. The end of an attack of paroxysmal tachycardia. Heart rate during attack 150 per minute. Heart rate after attack 86 per minute. The change in heart rate at the end of the abnormal rhythm is abrupt and there is a post-paroxysmal pause. The P-wave is inverted during the abnormal rhythm and occurs between R and T of the ventricular complex. There is a positive venous pulse during the paroxysm. The cardiac mechanism is diagramed below the tracing. The heterogenetic character of the ectopic rhythm is represented in the diagram by the white centers of the auricular rectangles which correspond to the inverted P-waves.

being represented by the white centers of the rectangles which correspond to auricular systoles.

Auricular Flutter.

Auricular flutter differs from paroxysmal tachycardia of auricular origin in the following particulars: (1) The auricular rate is more rapid and lies between 200 and 370 per minute. (2) Either because of the very rapid auricular rate alone or because of a depression of the conductivity of the junctional tissues in addition the ventricles do not respond to every auricular contraction. When the patient first comes under observation the ventricular rate is most often one-half the auricular. Vagus stimulation does not influence the auricular rate but slows the ventricles by increasing the grade of block. Digitalis has a

similar effect and it is common after the administration of this drug to find that the ventricular rate has been reduced to one-fourth of the auricular. In large doses digitalis frequently transforms auricular flutter into auricular fibrillation and finally this may be followed, when digitalis is discontinued, by the return of the normal rhythm. (3) Attacks of auricular flutter are usually much longer than those of paroxysmal tachycardia, their duration being measured in months or years rather than in hours or days. The essential features of this disorder are shown in Fig. 2. The auricles of the patient from whom this record was obtained were beating at the rate of 240 per minute while the ventricles which responded to every fourth auricular contraction were beating but 60 times per minute. Occasionally the ventricles responded irregularly to every third, fourth, fifth, or sixth auricular systole. The P-waves of the electrocardiogram have an abnormal outline indicating that this abnormal rhythm is ectopic. Each P-wave is accompanied by an a-wave in the venous pulse. The auricular rate was very constant from day to day and was not influenced by vagus stimulation. This fact together with the very rapid rate of impulse formation indicates sufficiently well the heterogenetic character of this ectopic rhythm.

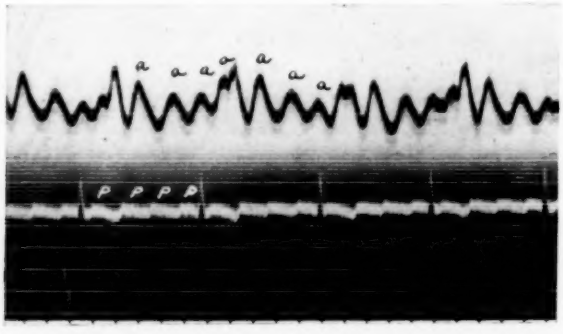

Fig. 2.—Lead II. Auricular flutter. The auricular rate is 240 per minute, the ventricular rate 60 per minute. The P-waves are abnormal in form. Corresponding to each P-wave there is an *a*-wave in the venous pulse.

Homogenetic Ectopic Rhythms.

Homogenetic ectopic rhythms are recognized by the following characteristics: (1) They are of comparatively slow rate. (2) The change in heart rate at their onset and at their end is gradual and is usually not very marked. (3) The rate of these rhythms is influenced like the normal rhythm by exercise and by stimulation of the extracardiac nerves. (4) There is no pause following the abnormal rhythm similar to that seen at the end of an attack of paroxysmal tachycardia.

Ectopic rhythms of this type lie dormant everywhere in the heart although practically we have to deal only with those centers which lie in the specialized tissues of the atrioventricular junction whose rhythmicity is greater than that of the undifferentiated cardiac muscle elsewhere. All of these centers remain quiescent until an increase in their rhythmicity or a decrease in the rhythmicity of the sinus-node enables that one whose inherent rate is highest to assert itself. Since most homogenetic ectopic rhythms have their origin in the junctional tis-

sues they are usually referred to as atrioventricular rhythms. Such rhythms may arise spontaneously, most often during the course of certain acute diseases especially diphtheria[2] and acute rheumatic fever.[3] More often they may be produced by stimulation of the vagus.[4, 5] They may also occur after the administration of digitalis.[6] We have recently been able to show that such rhythms may be produced in the majority of young adults by vagus stimulation between eight and fifteen minutes after the administration of one-fiftieth of a grain of atropin sulphate.[7] This drug appears to increase the susceptibility to atrioventricular rhythm by a selective action upon the vagus endings in that it paralyzes those that are found in the auriculoventricular node before it paralyzes those that lie in the sinus node. We have been able to obtain a large number of atrioventricular rhythms by this method. They are usually of short duration and are

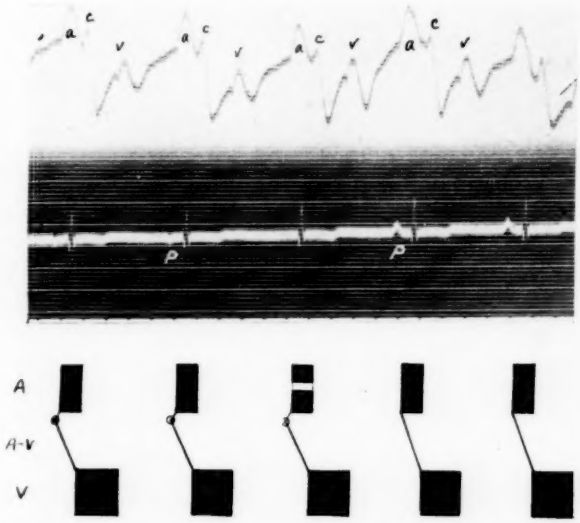

Fig. 3.—Lead III. An A-V rhythm of type 1, with the transition to the normal rhythm. During the A-V rhythm the P-wave is inverted and the P-R and *a-c* intervals are slightly reduced. In the third cycle the P-wave is invisible although there is a definite *a*-wave in the venous pulse. The absence of the P-wave is due to the fact that two excitation waves met in the auricular walls and neutralized each other electrically. The cardiac mechanism is diagramed below the tracing. This A-V rhythm originated high up in the junctional tissues or possibly in the coronary sinus region.

not accompanied by symptoms except that palpitation is occasionally experienced when auricles and ventricles contract simultaneously. For convenience in their description atrioventricular rhythms may be divided into three types; type 1 in which the P-R interval is present but reduced, type 2 in which the P-R interval is zero, and type 3 in which there is an R-P interval.

Atrioventricular rhythm of type 1 is illustrated in Fig. 3. The abnormal rhythm is comparatively slow, there is no abrupt change in heart rate and no pause at its termination. These facts indicate its homogenetic character. The P-wave of the electrocardiogram is inverted and the P-R and *a-c* intervals are both slightly reduced indicating that the ectopic rhythm originated in the upper levels of the junctional tissues. The cardiac mechanism is diagramed below the tracing.

Atrioventricular rhythm of type 2 is illustrated in Fig. 4. This ectopic

rhythm displays the same characteristics as that of Fig. 3 except that the P-wave cannot be made out during the abnormal rhythm. There is a tall wave in the venous pulse, however, which indicates that auricles and ventricles contracted simultaneously. The P-wave is buried in the R-wave and the P-R interval may therefore be given as zero. Such rhythms arise in the central portion of the junctional tissues.

Fig. 5 illustrates atrioventricular rhythm of type 3. Here again the abnormal rhythm has the characteristics which place it in the homogenetic group. In this instance, however, the P-wave which is inverted falls behind R and there is an R-P interval of .175 seconds. Such rhythms originate in the lower levels of the junctional tissues.

The Transitions between Normal and Atrioventricular Rhythm.

The transitions between normal and atrioventricular rhythms are of interest since they illustrate very well the essential differences between homo-

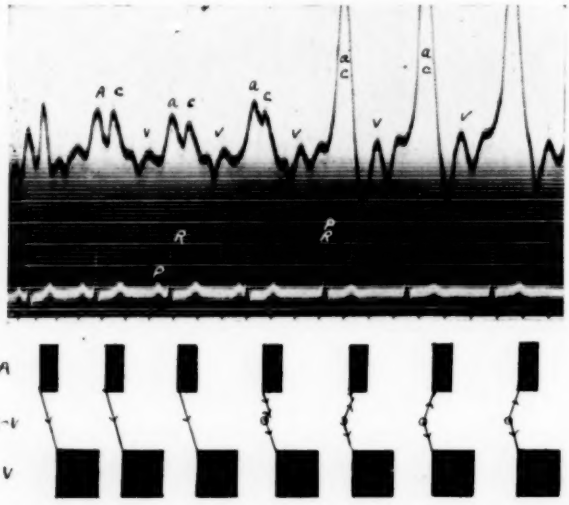

Fig. 4.—Lead II. An A-V rhythm of type 2, with the transition from the normal rhythm. At the beginning of the abnormal rhythm P without changing its form gradually approaches and disappears within the R-wave. During the abnormal rhythm (last three cycles) P is invisible but there is a tall wave in the venous pulse indicating that auricles and ventricles contracted simultaneously. This rhythm originated in the central portion of the junctional tissues, presumably in the region of the A-V node. The cardiac mechanism is diagramed below the tracing. Note that in the fourth and fifth cycles there is an interference of two excitation waves in the junctional tissues and that in these two cycles the auricles responded to the sinus node and the ventricles to the center in the junctional tissues.

genetic and heterogenetic ectopic rhythms. A transition from atrioventricular rhythm of type 1 to the normal rhythm is shown in Fig. 3. There is very little change in heart rate at the end of the abnormal rhythm. The P-wave of the third cycle which marks the transition from abnormal to normal rhythm is isoelectric. In this instance the two excitation waves, one from the sinus node and one from the junctional tissues, neutralized each other electrically. More often the P-waves which are due to the interference of two oppositely directed excitation waves in the auricular muscle are either small and upright or diphasic (Fig. 5).

When transitions from normal rhythm to A-V rhythm of type 2 occur (Fig. 4) the P-wave of the normal rhythm does not change its form but there is a gradual shortening of the P-R interval so that P gradually moves up to and disappears in R. No deformed or inverted P-waves are seen since the excitation wave from the junctional tissues does not reach the auricles until the beginning of the R-wave. The shortening of the P-R interval with no change in the shape of the P-wave which occurs during such transitions indicates that for a short period the auricles respond to the sinus node while the ventricles respond to the atrioventricular node.

This period of auriculoventricular dissociation is much longer as a rule in transitions from the normal rhythm to an atrioventricular rhythm of type 3. In Fig. 5 it will be seen that the P-wave gradually approaches the R-wave and finally appears after it without changing its form, indicating that during this period the auricles are responding to the sinus node as in the normal rhythm. In the seventh cycle, however, the P-wave is deformed because in this instance there was an interference of two excitation waves in the auricular muscle. After this transitional P-wave auricular systole is represented by an inverted P which means that in the last two cycles seen in the figure the auricles responded

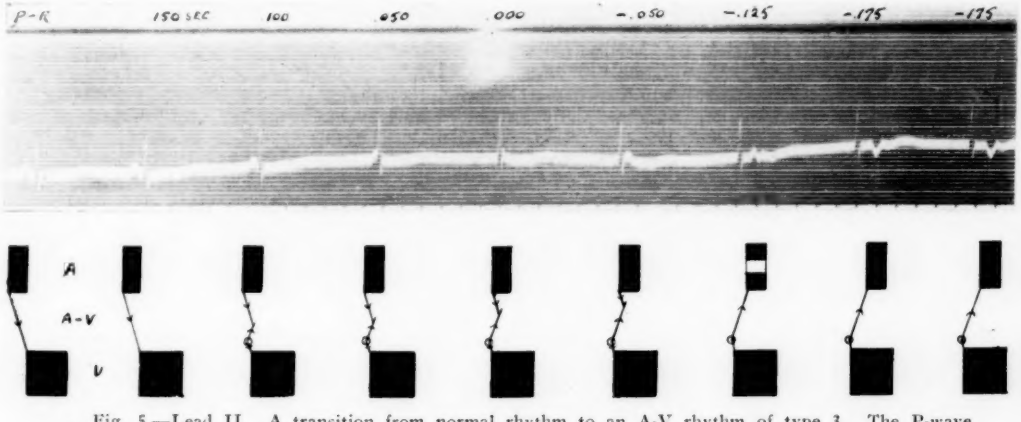

Fig. 5.—Lead II. A transition from normal rhythm to an A-V rhythm of type 3. The P-wave gradually approaches R and finally appears after R in the sixth cycle without changing its form. From cycle three to cycle six the auricles responded to the sinus node while the ventricles responded to a center low down in the junctional tissues, probably in the main stem of the His bundle just above its bifurcation. The two excitation waves met during this time in the junctional tissues. In cycle seven the two excitation waves met in the auricular walls and the P-wave of this cycle is deformed. In the last two cycles the auricles responded to the junctional tissues and the P-wave is inverted. There is an R-P interval of .175 seconds during the A-V rhythm while the normal P-R time is only .150 seconds in this case.

to the center in the junctional tissues. When the transition from normal to A-V rhythm is not complete short periods of dissociation similar to that seen in Fig. 5 may occur alone. In such cases the fact that the P-wave is of the normal form while the P-R interval is reduced should lead to their correct analysis.

The Form of the P-Wave and of the Ventricular Complex in Atrioventricular Rhythm.

From the analysis of a large number of atrioventricular rhythms obtained by vagus stimulation after the administration of atropin we have found that in

A-V rhythm of type 1 the P-wave is inverted in leads II and III and upright in lead I. The most marked inversion occurs in lead III. These observations indicate that the average direction in which the excitation wave travels over the auricles is upward and to the left rather than downward and to the left as in the normal rhythm. This makes it seem likely that A-V rhythms of type I, originate in the region of the coronary sinus. In A-V rhythm of type 2 the P-wave occurs simultaneously with R and is invisible in all leads. In A-V rhythm of type 3 the P-wave is inverted in leads II and III. In the single instance in which we have obtained an A-V rhythm of this type in lead I the P-wave was invisible. This was also the case in an A-V rhythm of type 3 described by Williams and James[8] and in another case described by White.[9] It seems likely therefore that this is the rule and that the excitation wave does not follow exactly the same course over the auricles in A-V rhythms of type 3 that it follows in those of type 1.

The ventricular complex in A-V rhythm is usually of the normal type since the excitation wave is distributed over the ventricles by the same route as in the

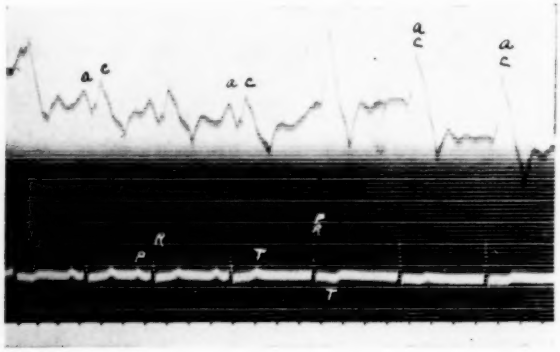

Fig. 6.—Lead III. An A-V rhythm of type 3 from the same patient as Fig. 4. The ventricular complex is abnormal during the A-V rhythm in that R is taller and is not notched and T is inverted.

normal rhythm. Occasionally, however, the ventricular complexes are distinctly abnormal during an A-V rhythm of type 2 or 3. This may occur in normal individuals and is most common in lead III. In Fig. 6, for example, the ventricular complexes of the ectopic rhythm are distinctly different from those of the normal rhythm; the R-wave is taller and is not notched and the T-wave is inverted. Since the origin of the A-V rhythm in this case was well above the bifurcation of the His bundle as is shown by the fact that there is no R-P interval the abnormality of the ventricular complexes can we believe best be explained by assuming that the center which gave rise to the ectopic rhythm was so situated in the A-V node that the excitation wave did not pass through the lower junctional tissues by the normal route and therefore did not pass down both branches of the His bundle simultaneously.

The Idioventricular Rhythm of Complete Heart-block.

In complete heart-block the stimuli which are formed at the sinus node are prevented from reaching the ventricles and the most rhythmic of the ventricu-

lar centers acts as pacemaker for these heart chambers. The idioventricular rhythm in such cases is a slow one, usually about 30 per minute. Its rate is influenced by exercise but not as a rule by vagus stimulation. The rhythm is a homogenetic one: its failure to be influenced by vagus stimulation is probably due to the destruction or functional depression of the vagus fibers that go to the ventricles by the same agent which produced the heart-block.

The ventricular complexes of the electrocardiogram in complete heart-block is usually of the normal form indicating that the idioventricular pacemaker is situated in the junctional tissues below the block. Occasionally, however, the

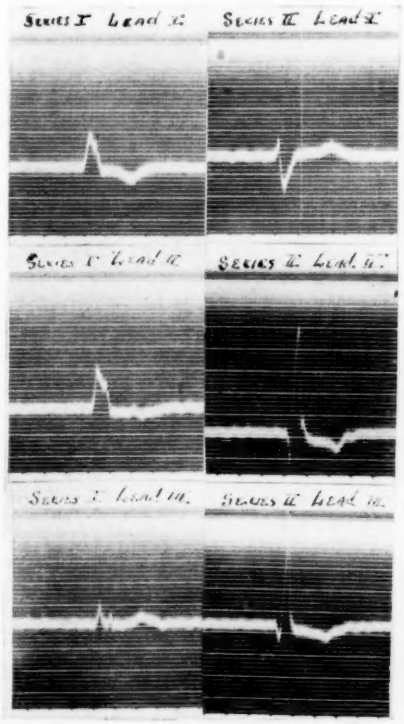

Fig. 7.—Two distinct types of ventricular complex obtained from a case of auricular fibrillation with complete heart-block. The type of ventricular complex labeled Series II was the one usually present. (See discussion in text.)

ventricular complexes are abnormal and such cases must be explained by assuming that the controlling center lies below the bifurcation of the His bundle or that if the center lies above this point there is a block in one branch of the His bundle. Still more rarely the type of ventricular complex in complete heart-block changes from time to time and when this occurs one must assume either that there are two centers competing for the position of ventricular pacemaker and that now one and now the other fills this post or that there is a single center and that the changes in the form of the ventricular complex are due to variations in the conductivity of the branches of the His bundle. It is usually impossible to decide which of these two explanations is the correct one. An ex-

ample of complete heart-block in which two types of ventricular complex were observed is given in Fig. 7.

Summary.

In conclusion we may say that we believe Lewis' division of ectopic rhythms into two groups, homogenetic rhythms and heterogenetic rhythms, is in harmony with the facts and that it is of the greatest importance since it distinguishes between ectopic rhythms which are essentially pathologic and those which are essentially physiologic in origin. The principal differences between the two types of ectopic rhythms are tabulated below.

HOMOGENETIC.	HETEROGENETIC.
1. Slow rate.	1. Very rapid rate.
2. No pause at end of abnormal rhythm.	2. Post-paroxysmal pause.
3. Slowed by vagus stimulation. Rate increased by exercise.	3. Usually not affected by vagus stimulation or exercise.
4. No marked nor abrupt change in heart rate at beginning and end of abnormal rhythm.	4. Abrupt and marked change in heart rate at onset and end of abnormal rhythm.
5. Practically always originate in junctional tissues.	5. Usually originate in auricular muscle, rarely in junctional tissues.
6. Essentially physiologic in origin.	6. Essentially pathologic in origin.

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THE EFFECT OF VENOUS STASIS ON THE PROTEINS OF HUMAN BLOOD SERUM*

By Albert H. Rowe, M.S., M.D., San Francisco, Calif.

THAT mechanical increase in the blood pressure does not increase the concentration of arterial or venous blood was shown by Böhm[1] and Asher.[2] On the other hand, it has long been known that venous stasis does increase the concentration of the blood. Grawitz,[3] Schultz and Wagner[4] and many others have studied the increase in the erythrocytes, hemoglobin, specific gravity, and total solids of the whole blood that results from stasis.

The total protein of serum also increases with stasis and the estimation of this increase gives a less erroneous idea of the amount of stasis present than does a determination of the concentration of the whole blood. This fact is shown in many cases of cardiac failure where there may be an increase in the red count and hemoglobin, as well as in the specific gravity and total solids, while the serum is definitely thinner than normal. An explanation of this apparent discrepancy is afforded by the work of Cohnstein and Zuntz[5] who showed that with a constant number of red cells in the circulatory system, their number in any blood vessel depends on the width of that vessel. Thus narrow capillaries hold relatively much plasma and few red cells, which forces extra reds into other parts of the circulatory system. It can be seen, therefore, that changes in the width of vessels in disease can alter the true relation of red cells to plasma. Moreover Böhme[6] has shown that the red count of capillary blood is increased above normal with venous stasis while the concentration of the serum is not changed. The same holds true with the red count and the serum when cold is applied to the skin, as was shown by Grawitz,[3] Winternitz[7] and Nonnenmacher.[8]

Kreibich,[9] while doing refractometric determinations of total protein in the blood sera, noticed accidentally that venous blood which had been under moderate stasis showed a higher refractive index than did arterial blood but he failed to recognize the cause. To Schwenker,[10] working under Böhme,[6] belongs the credit of working out the fact that the total protein of the blood serum progressively increases with the degree of venous stasis. For the determination of the proteins the refractometric method of Reiss[11] was used. Some of his data have been incorporated in articles by Reiss and Böhme together with some of their individual observations on this same problem. Schwenker definitely showed that the longer stasis was sustained the higher was the increase in total protein. Thus he found that in 16 minutes there was an increase from 7.4 per cent protein to 11.4 per cent while in 42 minutes the increase was from 7.2 per cent to 12.16 per cent. His method was to produce stasis with the inflated arm band of a blood pressure machine, the pressure being held stationary throughout the experiment at a point just a little above the diastolic pressure as advised by Zur Verth,[12] in order to give the most intense stasis. A

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lumbar puncture needle with stylet was inserted into the median vein before stasis was begun and was left throughout the experiment. When a specimen of blood was taken the stylet was withdrawn from the needle's bore and reinserted when enough blood was obtained. Schwenker also determined that even the physiological stasis produced by hanging the arm over the bedside, as often occurs in sleep, gave an increase of .7 per cent protein in nine minutes.

Cohnheim's[13] explanation of this increase in total protein due to venous stasis still holds. His assumption was that a protein-poor fluid passes out

NAME	AGE	DIAGNOSIS	DURATION OF STASIS	DEGREE OF STASIS	ALBUMIN		
					NO STASIS	WITH STASIS	INCREASE DUE TO STASIS
1. G. C.	21	Cardiac neurosis	1 min.	Tourniquet	5.7		
2. H.O.M.	50	Arteriosclerosis Chronic interstitial nephritis	1½ min.	"	5.3	5 6	.3
3. F. G.	50	Fractured femur and fibula	1¾ min.	"	4.4	4 6	.2
4. Z. M.	17	Chronic nephritis	8 min.	75mmHg.	3 85	4 2	.35
5. E. L.	40	Gall stones Arteriosclerosis	10 min.	100 "	4.8	6.1	1.3
6. I. R.	53	Duodenal ulcer	10 min.	100 "	5.	6.2	1.2
7. J. I.	35	Pulmonary tuberculosis. Hyperthyroidism	13 min.	90 "	4.6	5.8	1.2
8. J. B.	41	Hysteria	15 min.	100 "	5.	7.6	2.6
9. P. M.	34	Subacute bronchitis	15 min.	100	5.7	7.65	1.95
10. M. S.	40	Constipation	15 min.	100	5.2	7.1	1.9
11. W. B.	43	Dyspepsia	15 min.	100	5.4	7.9	2.5
12. HOM	50	Arteriosclerosis Chronic interstitial nephritis	20 min.	120	5.3	8.2	2.9

The normal values for albumin, globulin and nonprotein as determined by Robertson's method were recently published by Tranter and Rowe.[22]

through the capillary walls into the surrounding tissues and finally into the lymph spaces which consequently increases the concentration of the serum in the veins and also the amount of lymph in the lymphatics, both of which effects were shown by Schultz and Wagner to occur with stasis. Water and salt pass in and out of the blood through the capillary walls with the greatest ease. Protein has this same power to a much less extent, which is well demonstrated

in a research by Morawitz[14] in which he showed that after large bleedings in animals the refractive index rose towards the normal value within a few hours, showing that large amounts of protein must have gone through the capillary walls into the blood stream. The same thing is shown by the older work of Magnus[15] who proved that the protein content of the blood underwent definite changes when large amounts of physiological salt solution were injected intravenously.

This increase in the refractive index resulting from stasis is, in a small

GLOBULIN			TOTAL PROTEIN			NONPROTEINS	
NO STASIS	WITH STASIS	INCREASE DUE TO STASIS	NO STASIS	WITH STASIS	INCREASE DUE TO STASIS	NO STASIS	WITH STASIS
2.			7.70	7 87	.17	1.3	1 3
1.9	2.	.1	7.2	7.6	.4	1.3	1.3
2.7	2.8	.1	7.1	7.4	.3	1.1	1.1
2.3	2 8	.5	6.15	7.	.85	1.4	1.4
3.1	3.6	.5	7.9	9.7	1 8	1.1	1.1
2.3	2.6	.3	7.3	8.8	1.5	1.2	1 2
2.5	2.9	.4	7.1	8.7	1.6	1.3	1.3
2.4	3.1	.7	7.4	10.7	3.3	1.2	1.2
2.	3.24	1.24	7.7	10.89	3.19	1.3	1.3
2.	2.6	.6	7.2	9.7	2.5	1.	1.1
2.1	2 7	.6	7.5	10.6	3.1	1.4	1.4
1.9	3.3	1.4	7.2	11.5	4.3	1 3	1.3

but definite measure, due to the increased CO_2 contained in the blood. Koranyi and Bence[16] have found that the refractive index of serum is increased when CO_2 is passed through the whole blood. The cause of this increase was shown by Hamburger [17] to be due to the passage of water and chlorides from the serum into the erythrocytes along with the passage of certain organic substances from the red cells into the serum. Schwenker and Böhm found that in mild stasis the CO_2 increases the refractive index to an extent equal to .1 per cent protein

while in very severe artificially produced stasis the CO_2 may cause an increase equal to from .2 to .42 per cent protein. No definite differences could be made out by these authors between normal venous and capillary blood, showing that the amount of CO_2 that physiological venous blood contains does not influence the refractive index.

The question arises whether the stasis occurring in diseased conditions would make the refractive estimation of total proteins useless. When the venous pressure is higher than normal throughout the whole circulation, as Movitz and Tabora[18] as well as other investigators have shown is the case in heart insufficiency with general edema, the refractive index of venous and arterial blood would differ very little. But when marked edema for example is present in the arm, from a vein in which blood is taken, it is entirely possible that the index will be higher, though only to a slight degree, than that of the unobstructed arterial blood. That this is probable was shown by Achard and Demanche[19] who found that the venous blood taken from an edematous extremity has a higher refractivity than blood taken from the same extremity after the edema has been pressed out, which procedure does away with the stasis caused by the extreme edema. This effect of stasis is not transmitted to the capillary blood except in rare cases since Böhme showed that serum from capillary blood taken from an arm under stasis yields the same refractive index as does the serum from the arterial blood of the same patient. Thus in order to ascertain if edema in the arm, from a vein in which blood is taken, is causing stasis which yields a comparatively large error, it is only necessary to see if the refractivity of the venous serum compares with that of the capillary serum.

The object of the experimental part of this paper has been to show how this increase in protein which occurs with stasis distributes itself in the albumin and globulin fractions and to watch the effect of this stasis on the non-protein elements. T. B. Robertson's[20] microrefractometric method has been used in all these determinations. All measurements have been made by the automatic pipette recently described by the author.[21]

To produce the desired stasis in the veins of the forearm, the arm band of a blood pressure machine was placed around the arm and the pressure was sustained at a constant level between the systolic and diastolic pressures. While the stasis was still active, about 10 c.c. of blood were drawn into a dry Luer syringe, which amount yields ample serum for proper controls. An equal amount was then taken from the other arm without causing any stasis. These specimens of blood were then allowed to clot completely and the serum was separated by centrifugalization. Since hemolysis increases the refractive index, no hemolyzed sera were used. The technic of Robertson was followed exactly except that .5 c.c. of serum instead of 1 c.c. was used in the protein precipitation with heat and acetic acid. Normal and pathological cases were used in order to determine if any difference in the partition of the increased protein between the albumin and globulin fractions occurred in either health or disease. The patients were all taken from the Out-Patient Department of the Massachusetts General Hospital. Since exercise increases the refractive index, all patients were allowed to rest quietly from ½ to 1 hour before blood was taken.

From the experimental data given in the accompanying table it appears:

1. That with a constant degree of venous stasis between the diastolic and systolic pressures, the total proteins increase with the duration of the stasis.

2. When blood was taken from a vein, one minute after an ordinary tourniquet was applied to produce stasis, there was only .17 per cent increase in the total protein and therefore too small an amount to split into albumin and globulin.

3. In all of the remaining experiments except the fourth one, the increase in albumin was greater than was the increase of globulin. Part of this difference is probably due to the excess of albumin over globulin than exists in the normal sera of the cases in this series.

4. The nonprotein elements underwent no change due to the stasis.

5. In the fourth case the globulin increase was greater than the albumin possibly due to the fact that the amount of globulin in the normal specimen was greater in relation to the albumin than in any other case of this series.

From this and other experiences with Robertson's method, it appears that certain precautions must be taken in order to get correct results.

1. In obtaining blood from a vein, it is important to draw it off within one-half minute and always within one minute after the application of the tourniquet.

2. The syringe must be dry so as not to dilute the serum obtained.

3. The sera which are hemolyzed cannot be used since the refractive index is increased by hemolysis.

4. The patient must be at rest, preferably reclining, for one-half hour before blood is taken.

Work is now in progress on the effect of hemolysis, diet, and exercise, as well as disease on the relation of albumin to globulin in the blood serum.

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THE PROTEIN POISON OF VAUGHAN*

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MEDICAL literature is filled at the present time with articles on anaphylaxis or protein sensitization, and the theories concerning this phenomenon are almost as numerous as the workers in this branch of modern medicine. It is not my intention to advance any theory concerning this, and I do not think it necessary to mention any of them at this time, except that of Vaughan,[8, 23] which is as follows:

Sensitization consists in developing in the animal a specific protelytic ferment, which acts upon the protein bringing it into existence, and no other. That is, if you inject a protein parenterally into an animal, such as the guinea-pig, the cells of this animal immediately set to work to get this protein out of the way. They accomplish this by digesting it by means of a ferment. This ferment in digesting the protein liberates a poison, which, however, is set free so slowly that the animal is not much affected. This ferment is produced rather slowly at first, but after a time the protein is all digested, and an excess of the ferment is stored in the body cells as zymogen. After this has taken place, a second injection of the same protein, and no other, will cause a liberation of this specific ferment and digestion of the protein with an immediate production of large quantities of poison sufficient, if dosage and time intervening between are properly regulated, to cause the death of the animal. This poison can also be prepared by chemical means, and it is with the properties and attempts at purification that this paper deals.

Chemical Production of Protein Poison.

The production of protein poison by chemical means was first reported by Vaughan[1] and Wheeler[2] in 1905; and their method with few minor variations has been followed in the preparation of the poisons with which I have worked.

A weighed amount of protein is placed in a flask, and shaken with twenty volumes of absolute alcohol containing 2 per cent of sodium hydroxide, in order to thoroughly mix the protein with the solution and avoid caking when the suspension is heated. This mixture is then heated in a water bath, with constant stirring, under reflex condenser at the boiling point of alcohol for one hour after the alcohol begins to boil; temperature of water in the bath being 86° to 92° C. The mixture is allowed to cool and is then filtered, keeping as much of the residue in the flask as possible. After the extract has been filtered off, the small amount of residue on the paper is returned to the flask, and the combined residues extracted with twenty volumes of absolute alcohol as before. In all, three extractions are made. This divides the protein into two portions, one soluble in the alkaline alcohol and very poisonous, but apparently nonspecific; the other insoluble in alcohol and specific. These extracts are combined, filtered if neces-

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sary, and then made slightly acid with hydrochloric acid. This precipitates practically all the sodium as sodium chloride, which is filtered off. The filtrate is evaporated to dryness, preferably in a partial vacuum. The resulting residue is a brownish powder, very hydroscopic and readily soluble in alcohol and water, and is the protein poison. When prepared in this way, the poison is known as the crude poison, and can be purified partially by dissolving again in absolute alcohol and filtering; this removes considerable quantities of sodium chloride. The filtrate from the salt is again evaporated to dryness in partial vacuum and is usually somewhat more toxic than at first. This is the ordinary routine method of preparing the protein poison, and is the one used in preparing most of the poisons that I have studied.

Action on Animals.

No definite chemical test having been found for the identification of this poison, all tests for its presence and amounts are necessarily physiological. Similarly as in experimental work in anaphylaxis, the guinea-pig reacts promptly and is the best of the common laboratory animals for this work. Vaughan in his text-book on "Protein Split Products," pp. 125-6, sums up the action of the poison on guinea-pigs so thoroughly that I can do no better than to repeat his observations here. "When doses of this powder are given intraperitoneally in amounts varying from 8 to 60 milligrams, according as to whether we have been careful to remove most of the common salt or not, a fatal result follows in guinea-pigs in from thirty to sixty minutes. Within fifteen minutes after injection the temperature begins to fall, and sometimes within half an hour has reached 94° F., or even lower. At first, after an interval of from five to ten minutes immediately following the injection, the animal appears restless, runs about the cage, and shows a great tendency to scratch itself, this undoubtedly being due to itching sensations in the skin, caused by irritation of the peripheral nerves. The animal then begins to show evidence of lack of coordination, which is rapidly followed by partial paralysis, which is especially marked in the hind extremities. This stage lasts for from five to ten minutes, during the latter part of which the animal usually lies quietly on one side. From this stage the animal passes into what one might term the convulsive stage. These convulsions are usually clonic in nature, and, as a rule, at first involve only the neck muscles, the head being momentarily drawn backward on the back. At first these convulsions are but slight in degree, and are separated by considerable intervals of time. Soon, however, they become much more frequent and of much greater severity. Gradually they become more and more general in their extent, until all the muscles in the body become involved in violent clonic convulsions. This stage when present presages a fatal outcome; rarely an animal recovers after reaching the convulsive stage. During a convulsion or occasionally in the interval of calm, respiration ceases. The heart, however, continues to beat, at first with perfect regularity and no acceleration; indeed, the rate seems to be somewhat slower than normal. Gradually the beat becomes more and more feeble, the rate and regularity being preserved to the end. It is usually only after an interval of from three to four minutes after the cessation of respiration that the heart

ceases to beat. As has been previously stated, a fatal issue, if it occurs at all, always results within one hour after injection, and usually within thirty to forty minutes. This is to a large extent independent of whether the dose is the minimum lethal dose or two or three times that amount. It is certainly entirely independent of the size of the pig.

"At autopsy no special gross lesions can be made out. The peritoneum is smooth and shiny throughout, and there is not the slightest evidence of either hemorrhage or even marked congestion in the omentum or mesentery."

The poison is acid in reaction (from 3 to 6 per cent acid calculated as hydrochloric acid), but this is only partly due to hydrochloric, since an aqueous solution of poison shaken on a machine with silver carbonate showed but a slight decrease in acidity after filtering, and no decrease in its toxicity. Fifty cubic centimeters of a water solution of which one cubic centimeter equaled 50 milligrams or a minimum lethal dose of the poison when given intraperitoneally to a guinea-pig, and with an acidity of 5.1 per cent calculated as hydrochloric acid was shaken for three hours with three grams of finely powdered silver carbonate. After filtering, the filtrate still killed in one cubic centimeter doses and showed an acidity of 4.7 per cent.

Action of Immiscible Solvents.

Ether removes a slight amount of waxy resinous material from acid solution of the poison or from solutions made slightly alkaline with either the fixed alkalies or ammonia. The material removed is practically nontoxic in either case. Chloroform, petroleum ether or benzine also remove small amounts of nontoxic material, as will amyl alcohol. An excess of acetone added to aqueous solutions of the poison precipitates a slight amount of nontoxic material. From the dry powdered poison contained in the thimble of a Soxhlet extraction apparatus, chloroform removes slowly, part of the poison, but the amount of this extract, after evaporation of the chloroform, necessary to kill is as much or more than the original poison.

Heavy Metals.

Many of the salts of the heavy metals give abundant precipitates with the protein poison. Some of the better ones for this purpose are the chlorides of mercury, copper, and platinum. Mercuric chloride precipitates only a small part of the poison from aqueous solutions. From solutions of the poison in absolute alcohol, mercuric chloride in absolute alcohol precipitates the greater part of the poison as an amorphous flocculent precipitate. On removal of the mercury from this precipitate by means of hydrogen sulphide, the residue after filtering and evaporation of filtrate in partial vacuum is much more toxic than before. If this residue is again dissolved in absolute alcohol and reprecipitated by mercury as before, the precipitate suspended in alcohol and mercury removed by hydrogen sulphide and filtrate evaporated as before, usually gives a still more toxic residue. I have been able in this way to obtain poison that would kill guinea-pigs in doses of .0005 grams, when given either intravenously or intracardiacly. But this is as far as I have been able to carry the purification by

this means. Usually when I get about this far, the toxicity begins to decrease, and continual reprecipitation by means of alcoholic mercuric chloride will not carry the purification any farther. All attempts to crystallize this mercuric precipitate have failed, and so, while I concede that this is one of the most efficient means of purification of which I know, it is a failure as a means of complete purification.

Copper chloride in an aqueous solution of poison gives no precipitate; however, in solution in absolute alcohol it will, providing the solution is not too concentrated. This precipitate, after removal of copper by hydrogen sulphide, is found to be very toxic, and I have been able to obtain in this way poisons that kill guinea-pigs in .0005 gram doses when injected intravenously. However, this precipitate, either before or after removal of the copper, is amorphous and cannot be crystallized. It was found impossible to determine the chemical composition of the poison in this way.

Platinic chloride precipitates part of the poison from either aqueous or alcoholic solution, but in either case before or after the removal of the platinum, the poison cannot be crystallized.

Although the most potent poisons have been obtained by precipitation with the heavy metals mentioned, the nature of the poison could not be determined. The precipitates of the poison with mercury and platinum in alcoholic solution are insoluble in any of the common solvents. The copper precipitate is nearly insoluble in alcohol, is absolutely insoluble in the other common solvents except water, which dissolves it readily. Attempts were made to purify this copper precipitate by dialysis, but were unsuccessful, as were all other attempts to crystallize it.

Dialysis of the free poison in aqueous solution shows that the poison diffuses slowly through collodium membranes. However, the time required for dialysis is so long that the poison obtained in this way is no purer than the original, so this cannot be used as a means of successful purification.

β-Imidazol-Ethylamine.

Dale and Laidlaw[17] in their work on the physiological action of the compound obtained from histidine by removal of carbon dioxide and known as β-imidazol-ethylamine (this compound will be referred to as β-I.) noted the similarity of the action of this compound to anaphylactic shock, which is similar to if not identical with the action of the protein poison.

There are indeed many points of similarity between the action of β-I. and the protein poison. For example, both cause contraction of the bronchioles, and death results from asphyxia. Both lower blood pressure in dogs.[11, 12] The fatal dose of β-I. for the guinea-pig when injected intravenously is .0005 gram, which is the same as required of our purest poison. Another striking point of similarity is the fact that a nonlethal dose of either will protect an animal against a lethal dose of the same given shortly afterwards. However, points of difference are practically as numerous as points of similarity. Protein poison is slowly destroyed by heat, particularly if any alkali or mineral acid is present, while β-I. is not. Indeed, one of the final steps in its preparation consists in heating with

concentrated hydrochloric acid.[15, 20] Protein poison can be removed from aqueous solution by simple salting-out processes, as I shall show later. In order to fully satisfy myself on this point, I have many times tried to separate the compound β-I., histamine, or ergamine, as it is sometimes called, and have always failed to do so.

The method used was that of Kossel and Kutscher,[24, 25] and is the same as Barger and Dale[18] used in the separation of β-I. from the intestinal mucosa, and Ackerman,[16] in its separation from bacterial cultures. I shall describe later a slight modification of the method of preparing protein poison, whereby I have been enabled to obtain sufficient poison from ten grams of casein to kill 8,000 guinea-pigs when given intravenously. Calculating the fatal dose of β-I. intravenously as .0005 gms., this would mean, if β-I. were the active principle of protein poison, that casein contains over 40 per cent of histidine. It seems that this at once precludes any possibility of β-I. being the protein poison, since casein contains less than 3 per cent of this amino acid.[24]

The possibility of the poison being a choline derivative has been considered, since atropin protects against either, but I have been unable to find any trace of neurine or similar compounds by any of the methods ordinarily used, and heating with baryta water soon destroys the protein poison, whereas it is one of the steps in the isolation of the choline derivatives.[22]

Amino Acids and Their Products.

The end-products of protein hydrolysis are the amino acids, and the extraction of proteins by means of 2 per cent sodium hydroxide in absolute alcohol is one method of hydrolysis. A study of the amino acids obtained from protein poison was reported by Wheeler in 1909,[26] but did not throw much if any light on the chemical constitution of the poison. With the idea in mind that perhaps some of the amino acids either before or after treatment with the alkaline alcohol might be poisonous, the following experiments were carried out:

One hundred milligrams of leucin were dissolved in water and injected intraperitoneally into a guinea-pig. The animal was unaffected. One hundred milligrams of leucin were heated on a water bath with 300 c.c. of alkaline alcohol for three hours, filtered, and the filtrate made acid with hydrochloric acid. The sodium chloride was filtered off, and the filtrate was evaporated to dryness, leaving 75 mgs. of residue. This residue was dissolved in water and injected intraperitoneally into a guinea-pig with no noticeable reaction. Glycocoll, alanin, histidine, tyrosin, phenylalanin, glutaminic acid, aspartic acid and tryptophane were tested in the same way and gave negative results. Many different mixtures of amino acids were tried, and also the products of these mixtures after treatment with alkaline alcohol, and in no case was any appreciable quantity of poison formed.

As an example of some of the mixtures that were tested in this way, the following experiment is recorded:

Fifty milligrams of each of the following were mixed intimately in a mortar: alanin, phenylanin, leucin, glycocoll, tryptophane, glutaminic acid, histidine, tyrosin, uric acid, and glucose. This mixture was then extracted in the usual way with three successive portions of 250 c.c. of absolute alcohol containing 2 per cent of sodium hydroxide. The extracts were combined, filtered, made slightly acid with hydrochloric acid and filtered. The filtrate was evaporated to dryness and the weight of residue found to be 370 mgs. One-half of this was dissolved in water and injected intraperitoneally into a guinea-pig, producing no reaction.

Action of Various Gases.

Carbon dioxide, oxygen, hydrogen or air do not appreciably affect the toxicity of the protein poison when passed through aqueous solutions, but hydrogen sulphide slowly lessens the toxicity.

Ozone gas prepared electrolytically from air and passed through aqueous solutions of poison at such a rate that each cubic meter of air contains 0.2 gms. of ozone slowly weaken the poison. It was found to be rather a difficult matter to treat solutions of the poison in this way, on account of the very energetic frothing that occurred. It was only by using a large number of bulbs, one above the other, somewhat on the plan of a reflux condenser about five feet high, and aspirating the ozonized air through by means of a water pump, that these solutions could be ozonized at all, and several days were required to complete the operation. It was found that it was possible to so ozonize solutions of the poison that they would still be poisonous, although the toxicity was decreased, and they would no longer give the Million reaction. The biuret test still persisted, however, and the question of whether or not the poison was a protein could not be settled in this way. The limits between the destruction of the ability to give the Million test and the complete destruction of the toxicity were very narrow. During the first two years of this work on protein poison, Witte's peptone was the protein usually worked with, and an attempt was made to ozonize this protein before extraction with the alkaline alcohol. For this purpose 100 gms. of Witte's peptone were dissolved in a liter of water and ozonized for a period extending over nearly four months. It should be stated, however, that the machine used to generate the ozone was continually getting out of order and required careful attention and frequent repair. The actual number of hours that the ozone was aspirated through this solution was 239. At the end of that time the solution was washed out of the apparatus, evaporated to dryness on the water bath and powdered; yield, 80 grams. This powder still gave a beautiful biuret test, but the Million was very faint, if given at all. It was soon found that this ozonized protein, which was very dark in color and possessed a very disagreeable odor, would no longer produce a protein poison when split up in the usual way.

Yield of Poison.

The amount of poison obtained from different proteins varies considerably. The germ substances of Vaughan, such as tubercle, typhoid, colon, and others, yield only about 30 to 40 per cent of poison by weight. With casein I have been able to obtain 90 per cent, and with peptone from 50 to 75 per cent. Of all the steps in the preparation of protein poison, the most important is that of neutralization. If the combined extracts are neutralized to phenolphthalein, but little poison is obtained, and enormous amounts are required to produce death. It has been my experience that if the alkaline alcoholic solution is made one per cent or more acid to phenolphthalein by means of hydrochloric acid, and the precipitated salt well washed with absolute alcohol, the largest yield of toxic substance is obtained. Another slight modification in technic that I have introduced in the preparation of this poison consists in not evaporating the acid al-

coholic solution to complete dryness and powdering it, but in evaporating under reduced pressure till the alcohol is all distilled off, and then taking up in water. Then of course if one desires to know the amount by weight, evaporate a measured portion to dryness at 100° C., cool in a desiccator and weigh. This obviates the necessity of long heating of the poison and produces a much more potent poison. In this way I have been able to prepare sufficient poison from ten grams of casein to kill 8,000 guinea-pigs when injected intravenously. There seem to be slight differences between the poison prepared in this way and the one prepared in the usual way. The most striking difference lies in the ratio of the fatal dose for the rabbit as compared to that for the guinea-pig. With the poison as ordinarily prepared, the ratio of dose for these two animals is practically proportional to body weight, usually about eight to ten times as much being required for ordinary rabbits as for guinea-pigs, when injected by the same route. With the poison prepared in this way, the amount necessary to kill rabbits intravenously is about seven hundred times as much as for guinea-pigs.

Some Serum Reactions.

Another remarkable thing is that if, after a rabbit dies following an intravenous injection of the poison and as much serum as possible is obtained, this serum is nontoxic fresh. However, after one hour in the incubator it becomes toxic for the guinea-pig; one cubic centimeter being sufficient to kill when injected intravenously, while the same amount is nontoxic if injected after fifteen, thirty, or forty-five minutes in the incubator.

On the other hand, if a nonfatal dose be injected into a rabbit and shortly after the rabbit be bled and the serum obtained, this serum is nontoxic either before or after incubation.

Normal rabbit's serum mixed with a solution of poison so that less than a fatal dose for the guinea-pig when given intravenously is contained in each cubic centimeter when placed in the incubator becomes toxic in about one hour. One cubic centimeter samples taken out every ten minutes and injected intravenously into guinea-pigs are nontoxic after ten, twenty, thirty, forty, or fifty minutes, but kill promptly after one hour.

Normal guinea-pig serum when treated in the same way becomes toxic after twenty minutes in the incubator, and the toxicity disappears after forty minutes.

Action of Alkalies and Acids.

Barium hydrate added to aqueous solutions of protein poison precipitates it completely. This precipitate is with difficulty soluble in water or physiological salt solution, but is readily soluble in alcohol or weakly acidified water. It is also readily soluble in barium hydrate solution and evidently forms a barium salt, since this new compound is readily soluble in water but insoluble in alcohol. The toxicity of the poison is weakened by contact with alkali and is soon destroyed by heating the barium compound.

A few drops of either acid or alkali added to an aqueous solution of the poison precipitate it as a gummy, resinous mass, and the toxicity decreases rapidly, particularly if heat is applied.

Salting-out Methods.

One of the best methods of purification that I have been able to discover consists in saturating the aqueous solution with sodium chloride. This precipitate is then dissolved in absolute alcohol to free it from excess of salt, filtered and evaporated. One very peculiar thing about this method is the fact that not alone is the poison increased in toxicity,—that is, a smaller amount is required to kill than before, but actually a greater number of fatal doses is obtained. For example, starting with 200 fatal doses for the guinea-pig when given intravenously, I have been able by precipitation in this way to obtain sufficient poison to kill over 300 guinea-pigs when given in the same way. This cannot be carried further, however, and reprecipitation of this by saturation with salt, while it never decreases the toxicity, fails to increase it as the first precipitation does. Just why a greater number of fatal doses can be obtained in this way, I am unable to say. I have failed to show that the filtrate from saturated salt solution has any marked protective action against the minimum lethal dose of the poison injected at the same time or subsequently to the poison injection. This filtrate contains slight amounts of poison and consequently if injected previously to the poison gives a slight protection for a few hours. However, the poison always shows this property. Many attempts were made to obtain a higher degree of purification of the poison by using varying concentrations of salt, but were unsuccessful.

As has been reported before,[26] magnesium sulphate and ammonium sulphate will also precipitate the greater part of the poison. Magnesium sulphate is not as effective as sodium chloride; and because of the solubility of ammonium sulphate in alcohol, this salt is not a good one to use, because if one attempts to remove it by dialysis, part of the poison is lost.

Conclusions.

1. All proteins contain a poisonous group.
2. The protein poison is not β-imidazol-ethylamine, although the physiological actions of the two are very similar.
3. The protein poison is not a choline derivative.
4. Protein poison is not a simple chemical compound, but is a protein change product, acid in reaction, capable of forming salts and reacting much like the globulins in its behavior to neutral salts.

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THE TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM*

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THE series of cases covered by this paper consists of 11 cases of tabes dorsalis; 1 of paresis; 1 of tabo-paresis and 1 of a psychosis of syphilitic origin thought by some to be early paresis.

The highest number of injections I succeeded in giving to a single patient was seven and the least was two. The average being four.

Eight cases of tabes and the early paresis were treated with the mercuralized serum as described by Byrnes.[1] One case of tabes was treated with serum alone while using the inunctions of mercury and one with the neosalvarsanized serum. The paretic was treated with neosalvarson directly into the spinal canal using as a diluent the spinal fluid and the tabo-paretic with serum alone while using the inunctions.

After January 1, 1915, I had to use the mercuralized serum entirely because of the difficulty of obtaining the salvarsan and can see no reason why this treatment should not be used while the supply of the latter is so limited.

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In all cases a Wassermann test was done on the remaining blood serum and a careful analysis of the spinal fluid after each injection. In this way all clinical improvement could be checked up with the changes in the spinal secretion.

The first thing that I noticed in using the mercuralized serum was what I called a severe reaction. The patients invariably complained of severe cramps in the legs, headache and nausea accompanied by a temperature of about 101°. It made me think that I had not prepared the serum properly and the internes were rather inclined to think that I was not warranted in continuing the treatments. However I did continue and soon discovered that in most cases the severe discomfort could be eliminated by heroin 1/12 gr. hypodermic at the time of the injection and a second one ten hours later if necessary. I also used gr. v each of pyramidon and aspirin every three hours for a day or two. This part of the treatment I soon learned to be most important if I wished the patient to continue the treatments. My early cases refused more than two injections because of the severe suffering so I was most happy to find a method for their relief.

The following case reports give the clinical and laboratory findings together with a few facts of interest from the history.

Case No. 1.—Mrs. A. Age 40. Her chief complaint consisted of shooting pains and and numbness in the legs and inability to walk well. She received two injections intradurally of 1/50 gr. of the bichloride and as the shooting pains and difficulty in walking improved so rapidly she left the hospital refusing further treatment. I saw this patient four months later when she stated that she had not improved any further and as she was not getting worse she chose not to take more treatments. The cell count on her spinal fluid decreased from 120 to 66 while all the other tests remained strongly positive.

Case No. 2.—E. F. Age 27. His chief complaint consisted of severe pain in the stomach and legs and inability to walk well. He had an operation for gastric ulcer 8 months previously with no relief. This patient was hard to handle and left the hospital after two treatments with no clinical improvement. All the tests on the spinal fluid remaining strongly positive.

Case No. 3.—Mrs. C. Age 42. Her chief complaint is of a continuous pain in her left arm and shoulder of one year's duration. This could not be accounted for except by the positive sign of tabes and the spinal treatments were given hoping to relieve it. She received three injections of 1/50 gr. of bichloride in serum intradurally with no change except that the cells in the spinal fluid dropped from 40 to 15. The reaction was most severe, temperature 102° and what was most distressing an uncontrollable nausea and vomiting that lasted a week each time. Accordingly she refused further treatments and left the hospital about April 1, 1915. The neuritis was unimproved. About three months later she returned to the hospital with a fracture of the humerus in the arm opposite to the one with the neuritis. Examination at this time showed no change in her condition.

Case No. 4.—G. D. Age 41. His chief complaint was due to a gummatous ulceration of the hard palate but he was given three intradural injections of the bichloride because he had all the signs of tabes although the latter was not giving him any trouble. He was given two doses of neosalvarsan and mercurial inunctions also. There were no changes in the tests on the spinal fluid or blood and at the end of two months as the gumma was entirely healed the patient ran away.

Case No. 5.—Mrs. M. F. Age 55. The chief complaint in her case was—pain in the legs and back, incontinence of urine and inability to walk. She was given four injections on general principles although the case was considered hopeless. The fifth treatment was refused because the pain in her legs was made worse but she volunteered the information that the numbness was much better. The spinal fluid was unchanged as was also her condition when I saw her four months later.

Case No. 6.—M. W. Age 54. His chief complaint consisted in paroxysmal pains over the heart and indefinite pain in the legs and back. He received five injections of the bichloride. The cell count decreased from 75 to 28 and the gold chloride reaction had improved, but the Wassermann remained strongly positive. Clinically he was better but refused further treatments because of the severe reaction.

Case No. 7.—Mrs. A. W. Age 38. She had been troubled with failing eyesight and inability to walk well for two years. This patient received six injections of the bichloride intradurally, 1.8 gm. of neosalvarsan intravenously, mercurial inunctions and potassium iodide for a period of four months at the hospital. The only improvement in the spinal fluid was a decrease in the cell count from 71 to 12. The Wassermann on the blood and spinal fluid remaining strongly positive. The clinical improvement was quite marked, both the eyesight and ataxia being much better. This patient was seen two months after the last treatment was given—her condition at this time being about the same as it was when she left the hospital.

Case No. 8.—B. E. Age 46. His chief complaint is as follows: pain and numbness in the legs, incontinence of urine, tight feeling about the chest, cough and expectoration. He received six intradural injections of the bichloride and although he had a severe reaction each time he continued the treatment because he knew he was getting better. After he had had four treatments he had a pulmonary hemorrhage from his tuberculosis and was allowed to go without a treatment for two months. He then received the last two treatments a month apart. The analysis of the spinal fluid and blood at the last treatment showed a cell count of 10 with all the other tests still strongly positive. This patient has been under observation for about eight months and has had the routine treatment with Ung. hydrargyrum. Clinically there was great improvement.

Case No. 9.—P. W. Age 37. His trouble consisted of great weakness, pain and numbness in his legs, incontinence and failing eyesight. He had the primary lesion but three years before. After seven injections of the bichloride the cell count dropped from 200 to 28, the Wassermann became negative 6 weeks before the Noguchi and the gold chloride was greatly improved. Clinically the patient was a well man and worked about the hospital as orderly during the last month.

Case No. 10.—L. H. Age 42. This man was diagnosed as a syphilitic pyschosis or possibly a beginning paresis and was troubled with headache, nervousness and an unexplainable desire to run his automobile into things. He admits having had a chancre 20 years before. His pupils are unequal but react to light and accommodation, knee jerks are lively, superficial lymph glands are palpable, the blood Wassermann faintly positive and all the tests on the spinal fluid are strongly positive for a syphilitic infection. The treatment here consisted of six intradural injections of the bichloride at intervals of two weeks, potassium iodide in increasing doses and daily inunctions of mercurial ointment. At the end of two months of treatment the blood Wassermann was negative and at the end of the series of injections the cell count had dropped from 40 to 14, the Wassermann had become negative, the gold chloride greatly improved and the globulin test but faintly positive.

Clinically there was great improvement in the mental condition of the patient who left the hospital soon after the last treatment and has not been heard of since.

Case No. 11.—Mrs. B. G. Age 45. Her complaint consisted in numbness of the legs and perineum, inability to walk well and slight incontinence of the urine and feces as well as cough and expectoration. A diagnostic spinal puncture showed a fluid under pressure with 20 cell, a positive globulin and Wassermann and a change in the gold chloride in the tabes region. The blood Wassermann was negative. The sputum contained many tubercle bacilli. She was given three intradural injections of neosalvarsanized serum and the spinal fluid became normal except for a positive Wassermann. The treatment was discontinued because of a pulmonary hemorrhage. After two months she was given the fourth and final treatment at which time I was surprised to find that the tests on the spinal fluid were all stronger than ever.

Clinically there was great improvement in her tabes and she refused further treatment. This case illustrates that the Swift-Ellis method causes the spinal fluid to approach normal rapidly, but two months without treatment allowed it to go back to where it was when I began the injections.

Case No. 12.—J. L. Age 64. The diagnosis here was paresis and emphysema and

the spinal fluid gave strongly positive tests for syphilis with 24 cells. Three injections of 3 mg. of neosalvarsan in patient's spinal fluid were given two weeks apart and as the patient gradually got worse the treatment was discontinued. At the time of the third treatment the cells had increased to 90 while the other tests on the fluid were still strongly positive. Two months after the last treatment the spinal fluid was again examined. No change was noted except that the cells had dropped to 14.

Clinically there was no improvement—in fact the patient seemed to be worse after each treatment.

Case No. 13.—J. N. Age 47. His complaint consisted of weakness and shooting pains in the legs and as his blood Wassermann was strongly positive he was put on daily inunctions of mercury. After 10 days of this treatment he received 12 c.c. of his own serum in 18 c.c. salt solution intradurally. A second injection of 15 c.c. of serum and 15 c.c. salt solution was given in 16 days. A third and last injection was given in 18 days. At the end of this time the cells had decreased from 58 to 20, the gold chloride had improved, the globulin and Wassermann remaining strongly positive. Clinically the pains had all left and the patient felt so well that he refused further treatments. He worked about the hospital for two months as orderly without a relapse and then left to be married.

Case No. 14.—C. S. Age 49. This patient had attacks of paralysis, an Argyll-Robinson pupil and exaggerated deep reflexes and was diagnosed paresis. She received four injections of increasing amounts of her own serum intradurally and daily inunctions of mercury. The cells fell to normal, the globulin was faintly positive, the Wassermann became negative and the gold chloride remained only slightly positive. The changes in the spinal fluid and the clinical improvement were so marked that I have always felt a little doubtful of this case.

In analyzing the cases it will be seen that those treated with the bichloride are arranged according to the number of treatments given. The greatest clinical improvement was seen in those receiving four injections or more.

Cases Nos. 1 and 2 receiving two treatments. No. 1 showing considerable and No. 2 no improvement whatsoever. Neither spinal fluid showed improvement.

Cases Nos. 3 and 4 received three treatments. No. 3 showing no change in the brachial neuritis, for which the treatments were given, while No. 4 believed himself completely cured but his feeling of well being came from the healing of the gumma. The spinal fluid was practically unchanged.

Case No. 5 received four treatments with no prominent clinical improvement nor important changes in the spinal fluid.

Case No. 6 received five treatments with some clinical improvement and a slight improvement in the spinal fluid.

Cases Nos. 7 and 8 received six injections. Both showed a great clinical improvement and considerable change in the spinal fluid.

Cases Nos. 9 and 10 showed the greatest clinical improvement and these were the only two that I succeeded in getting a negative Wassermann and Noguchi on the spinal fluid.

Cases Nos. 11 and 12 were started early last fall before the article by Byrnes was published and were continued as such for comparison.

Cases Nos. 13 and 14 received three and four injections respectively of their own serum after they had been using inunctions for about 10 days. Both cases showed considerable clinical improvement and some slight changes in the spinal fluid.

It had occurred to me that this latter method might be an efficient way of getting mercury into the subarachnoid space in combination with the specific

antibodies but Swift[2] recently has pointed out that remarkable changes can be produced by normal serum in some cases.

Although there is nothing startling in the results of this series there was enough clinical improvement to encourage me to continue the treatments and to conclude that patients with syphilis of the central nervous system should be given the benefit of some sort of intradural therapy. However when such good results can be produced with the Swift-Ellis method as reported recently by Smith[3] both clinically and in the spinal fluid it should be the method of choice. So long as the expensive salvarsan and neosalvarsan cannot be obtained then the mercuralized serum is certainly indicated.

HETEROPLASTIC BONE AND BONE-MARROW FORMATION ASSOCIATED WITH TUBERCULOSIS IN THE ADRENAL*

By Paul G. Woolley, M.D., Cincinnati, Ohio.

THERE are three hypotheses which are used in accounting for heteroplastic bone formation. One of these postulates the occurrence of embryonic "rests" of osteogenic tissue, misplaced during development, which in later years, under the stimulus of changed physiologic conditions, commence to grow and to produce bone. Another postulates the transference of osteoblastic cells by way of the blood stream by means of which they arrive in unusual situations where they lodge (embolism) and grow, producing bone. The third making use of the doctrine of metaplasia, holds that any cell may, within certain tentative conditions, change in its morphologic features, and become physically and perhaps chemically similar to other cells arising from the same general layer. To quote Adami—"while there may be conversion of one epiblastic or hypoblastic tissue into another epiblastic or hypoblastic tissue, and of one mesoblastic form of cell into another mesoblastic form, this conversion is of a limited extent; metaplasia of mesoblastic tissue into epiblastic or hypoblastic and vice versa does not occur."

The doctrine of metastasis and of metaplasia are the dominant ones. Of that which postulates embryonic rests there is no proof, save perhaps in the rarest instances and in teratomata. There is no evidence that such remains play any part in the production of heteroplastic bone in any of the usual cases. We believe that it may be practically discarded from this discussion.

The conception of metaplasia is the dominant one, combated though it be by certain pathologists such as Ribbert, who, though he admits physiologic metaplasia, nevertheless denies, at least to a very great extent, its occurrence under pathologic conditions. While we admit that there are a few, comparatively few, cases in which we can see no other explanation for the phenomenon than that held in the doctrine of metastasis (illustrated best by Le Count's case of Meta-

[2]Swift, H.: Jour. Amer. Med. Assn., 1915, lxv, 209.
[3]Smith, L.: Jour. Amer. Med. Assn., 1915, lxiv, 1563.
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static Osteomata), it seems to us that the logical explanation of practically all osseous heteroplasia can be found in the doctrine of metaplasia. Metaplasia, it seems, being a physiologic process of the widest possible application, must be also a phenomenon occurring under pathologic conditions. The tissues of the body are formed by gradually changing steps that appear in response to physical and chemical conditions. There is no intrinsic embryologic difference between the cells which are to form fibrous tissues and those which are to produce bone. The variations which arise are the results of physical and chemical differences in the environment of the cells which make it necessary that different structures be produced, and of the two orders of environmental factors, the chemical is more important. A fracture heals by fibrous union, not because there is no need for bone, nor because the stresses which might better be withstood by bone are absent, but because the chemical conditions, which demand bone, are absent.

If the proper nutritive conditions are present then only is bone formed, otherwise fibrous tissue of one or another order appears. In other words, there is no sharp boundary line between the various supporting tissues of mesoblastic origin, a fact which is best exemplified in the embryo during development.

How the necessary chemical conditions favoring osseous metaplasia are brought about, we do not know. My friend, Professor E. R. Le Count, has suggested that the presence of free blood plays a part, in what way I do not know. It is, however, an interesting fact that heteroplastic bone is most frequently associated with trauma and with inflammatory changes which are in turn associated with hemorrhage. It is possible that the presence of red blood cells which are colloids of a certain concentration and composition, furnish the proper physicochemical basis for deposition of salts in concentrations such that the mesoblastic cells during the growth associated with organization are impelled in the direction of bone formation rather than of fibrous tissue production.

The relationship between blood supply and bone formation has been commented upon by various investigators who have observed this type of metaplasia following ligation of the blood vessels of the kidney in which organ there seems a special unknown reason for the appearance of the process. In three out of four cases, after ligation of the renal vessels one may observe bone and medulla formation, and in the affected areas one sees practically the same process at work which takes place during normal ossification of membranes during development. (Sacerdotti and Frattin.) It has also been shown (Poscharissky) that in other organs than the kidney (liver, spleen, ovary, etc.) ligation is not followed by ossification, but only by necrosis and calcification. It seems as though there were an organ disposition to ossification. Poscharissky believes that the differences are due to different circulatory conditions, or to the fact that in some organs a longer time must elapse before ossification occurs. It is our experience that white infarcts of the kidney never show calcification. It is also our experience that mild degrees of calcification (i. e., deposition of small amounts of lime salts as granules in the renal cells) are not infrequent. Tying the efferent vessels of the kidney produces a condition of passive congestion, which is similar to red infarction, in which the red cells pass out into the tissues in large numbers, a condition quite unlike that found in white infarction. There seems

then to be at least a superficial relation between red blood cells and ossification in the kidney.

In the case which I wish to report, there was no evidence of hemorrhage. There was, however, an inflammatory process of a chronic nature which may or may not have been associated with emigration of red blood cells.

The patient, J. H., age 42, a white male, was admitted to the Neurologic Service of the Cincinnati General Hospital on March 13, 1915, complaining that he could not walk. He gave a history of malaria; and of smallpox, but of no other serious illnesses. For 20 years he had had an ulcer upon the right leg. For a number of weeks he had suffered from urinary incontinence.

The present illness began a few days before entrance to the hospital. One evening, he said, his legs began to feel strange. The next morning he had difficulty in walking to his work and finally was helped. He said his legs did not feel like legs and that they were weak. By the time he returned from work he was no longer able to walk.

Physical examination (according to the clinical history, which was obviously incomplete) showed "a well developed and nourished white man. Heart and lungs negative. Abdomen negative. Pupils and movements of eyes, negative. Muscles of the face, tongue and arms negative. Leg-knee jerks increased in both. Double Babinski. No ankle clonus. Legs are useless, but draw up involuntarily and then stay until drawn down. An ulcer nearly encircles (except at back) the right leg from the ankle nearly to the knee. Feces and urine passed involuntarily and frequently." Death occurred on April 11, 1915.

The clinical diagnosis was spastic paraplegia.

The postmortem was done 12 hours after death. The following is a copy of the protocol.

The body was that of a fairly well nourished white adult man 5 feet, 5½ inches long. The pupils were both somewhat dilated, the right more than the left. The skin of the face had a yellowish-brown tinge somewhat like that of Addison's disease. The conjunctivæ were pale. The eyes were sunken. On the inner side of the elbow joint (right) there were puncture wounds of needles. The chest was remarkably barrel-shaped, beside which there was a dorsal scoliosis. The abdomen was navicular. Age apparently 40.

Postmortem lividity was well marked. Rigor mortis was present and more marked in the arms and hands than in the feet. The right knee was slightly flexed. Midway between the sternal and axillary lines and 3 cm. below the intermammillary line, was a gaping wound which, except for the fact that its base was apparently (to the touch) cartilage, looked like the wound of an unhealed costotectomy. The edges of this seemed clean and healthy, and they were not undermined. The base was covered with a thin sero-purulent fluid. No sinus could be appreciated. On section it appeared that this lesion was one due to a destructive process in the 5th costal cartilage which had also involved the adjacent intercostal tissues. It did not involve the pleura. Eighteen cm. below the right knee and extending toward the ankle (12 cm. anteriorly, and ½ cm. posteriorly) was an ulcerated surface which extended completely around the leg. The margins of this were more or less irregular but were smooth and clean and showed every indication of indolence. The skin immediately about the edges was thickened, white and opalescent. Beyond this, both above and below, the skin and subcutaneous tissues were thickened and edematous, and the skin was pigmented. Such veins as could be seen above the ulcer were dilated and tortuous (varicose). The surface of the ulcer was smooth, red, moist, clean and indolent. The anterior lines of the tibiæ were smooth. There was no edema of the left foot or ankle. The penis and scrotum were cyanotic. Just to the right of the midline of the perineum, at a point where perineum and scrotum

joined, was the circular opening of a sinus which ran anteriorly under the skin of the scrotum. There was no evidence after dissection that this connected with the bladder or urethra, or with any other organ. On the back, scattered here and there, were very many irregular, some almost serpiginous, ulcerated surfaces which were for the most part covered with adherent fibrino-purulent pseudomembrane. This condition was especially well marked over the buttocks and in the sacral region. On attempting to turn the body on the side, a thin watery fluid containing flakes of mucous, ran from the mouth. The peripheral lymph glands were not noticeably enlarged. The teeth and gums were in a frightful state. The teeth, most of them decayed, were loose and hung loosely by their blackened stumps in pigmented suppurating gums. The subcutaneous fat was well developed. Internally (mesially) to the wound in the thorax was a small subcutaneous abscess containing a greenish-yellow caseous pus. It measured about 2 cm. in diameter.

The lungs collasped incompletely when the chest was opened. In the left side there were old anterolateral, posterior, and apical adhesions, and but a small amount of pleural fluid containing some blood. On the right side were numerous lateral, posterior, and apical adhesions and also a considerably increased pleural exudate. The lobes of the lungs were bound together by old adhesions. The surfaces of all the lobes of both lungs had a distinctly shotty feel which was due to the presence of innumerable miliary tubercles which were present, as section showed, everywhere throughout the pulmonary tissue. The lungs were quite edematous and moderately congested. Congestion was more evident in the left lung. At both apices were old apical scars. In the mediastinal fat and areolar tissue there was a considerable amount of black pigment which ran in lines down upon the upper surface of the diaphragm, where at one or two places it formed irregular patches. There was no evidence of thymus tissue. There was no increase of pericardial fluid. In the right heart was a mass of agonal (?) clot of considerable size. The valves were apparently normal except the aortic, upon the cusps of which were a few fine fibrinous vegetations. The aorta showed nothing else than a moderate degree of fatty degeneration with occasional areas of early atheroma. The spleen was large, soft and friable. The capsule was smooth and the edges rounded. The Malpighian corpuscles were just visible on the cut surface. The left kidney (225) was enlarged and pale. Its surface was irregularly, though only moderately, lobulated. The capsule was adherent. The surface was generally gray, mottled with lines of congested capillaries. The whole organ was moderately edematous. The glomeruli were not visible. The line of demarkation between cortex and medulla was not distinct. In the medulla at two points were small grayish nodules, which resembled miliary tubercles. The general consistency of the organ was increased.

The left adrenal was congested and showed evidence of increased lipoid change. A cortical adenoma was present in the medullary portion. The medulla was possibly hypertrophied.

The right kidney (110) was small and also irregularly lobulated. The middle third of the anterior half of the organ had all the appearances (externally) of the left kidney, but the rest of the organ was much paler and to the touch seemed to contain large cysts. On section it was discovered that practically the whole organ was the seat of a chronic suppurative process which had given rise to spaces filled with a cheesy, yellowish material. The pelvis, ureter and bladder showed no changes. The prostate was unusually friable and partly cystic, possibly tuberculous, though there was no good evidence of it. The seminal vesicles were apparently healthy. In the right epididymus was a focus of caseation. The testicles were normal. The right adrenal was small and cavitated. The liver was of about normal size (1,750 grms.). It was gray with a yellowish tinge, and marked with indistinct lines of congestion. There were the small tags of old adhesions upon its surface, especially on the right. Scattered here and there under the capsule were small rounded areas which resembled small tubercles. There were others of these within the substance of the organ. The cut surface was moist, gray and irregularly mottled. There was a large angioma in the right lobe (1×1.5 cm.). The bile ducts were patent, and there were no gall stones. There were old adhesions between the gall bladder and the gastro-colic omentum. The lymph glands in the celiac region were completely calcified and formed irregular masses. Scattered here and there in the mesentery, as a rule about halfway between the gut and the origin of that membrane, were calcareous masses irregular in outline, between which and the origin of the mesentery there were lines of hyaline fibrosis. The omentum was adherent to the parietal peritoneum at the

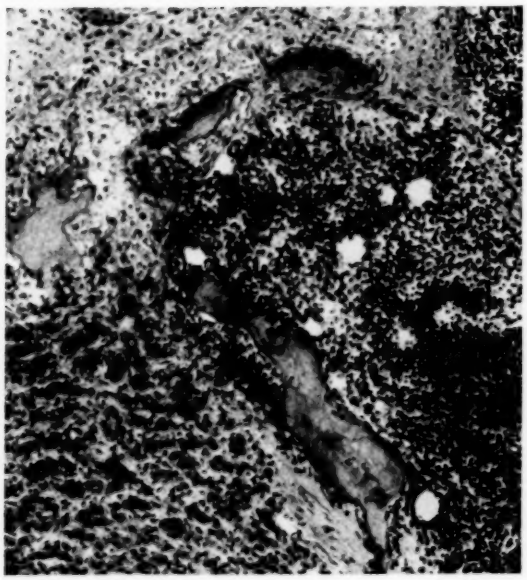

Fig. 1.—A section showing the presence of true bone and bone-marrow in the adrenal.

Fig. 2.—A section showing massing of small round cells about the bone in the adrenal.

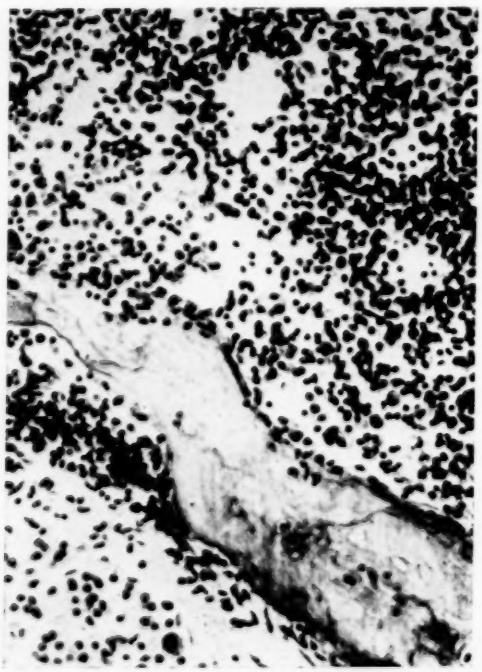

Fig. 3.—A section illustrating the same things as Figs. 1 and 2, but with greater magnification.

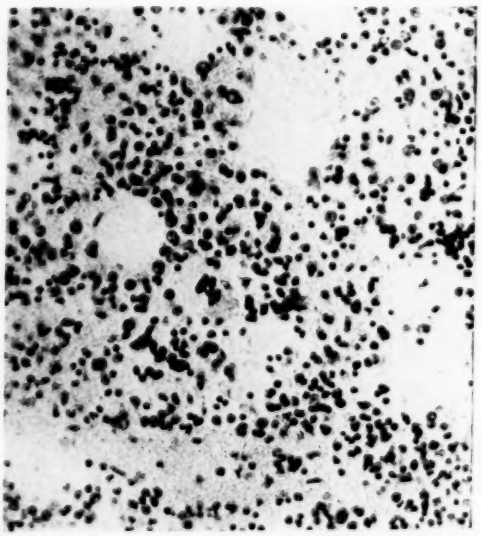

Fig. 4.—A section of the marrow in the adrenal, showing blood sinuses, and one megalocaryocyte.

right internal ring and at the right side of the bladder. The appendix was imbedded in a post-cecal pouch and folded sharply upon itself so that the tip and the origin were close together at the mouth of the pouch. The tracheal and bronchial lymph glands were enlarged, deeply pigmented and edematous. The thymus was absent. The trachea showed nothing abnormal. The intestine showed nothing remarkable. The brain (1,235 grms.) showed a marked diffuse edema, which was most evident over the vertex. In many of the sulci the fluid was clouded and in some places almost purulent. There was no distinct evidence of tuberculosis though there were occasional scattered areas which were suggestive. The ventricular fluid was increased. The brain was remarkably so. The cord also was very soft and almost diffluent. The pituitary was apparently normal. The pineal was edematous and had a calcified center.

The anatomic diagnosis was, generalized miliary tuberculosis; Tuberculous costochondritis; Acute vegetative aortic endocarditis; Tuberculous pyonephrosis;

Fig. 5.—A tuberculous area in the marrow in the adrenal.

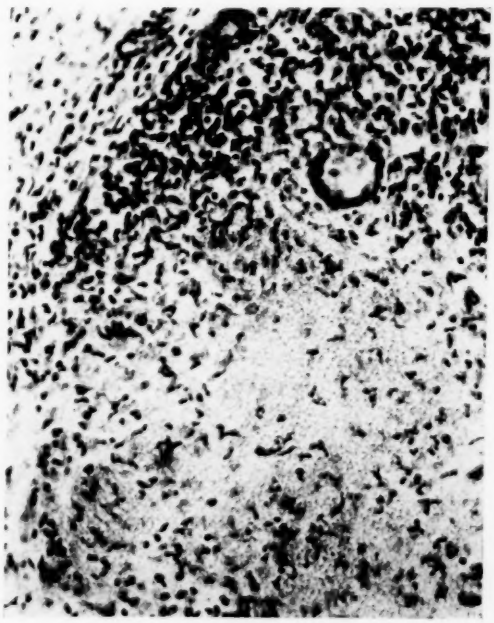

Fig. 6.—A tuberculous giant cell in an area in the adrenal.

Serous meningitis; Pyorrhea alveolaris; Varicose leg ulcer; Mediastinal anthracosis; Calcified mesenteric lymph glands; Adrenal tumor (?).

The histologic examination of the tissues preserved at postmortem confirmed the anatomic diagnosis, except in the case of the left adrenal in which region a most interesting condition was observed, and one which, so far as I have been able to discover, has been mentioned but a few times in medical literature.

The adrenal had been fixed in Zenker's fluid, washed, cut in thin slices, embedded in celloidin, cut and stained with hematoxylin and eosin (Delafield's) and in Mayer's acid hemalaun. In cutting the sections, the technician had realized that the tissue contained a small amount of something which grated upon the knife.

Upon examination nothing remarkable was encountered in the cortex. Aside from a moderate lipoid change, and the presence of a few foci of so-called ade-

nomas, there was nothing worthy of note, except at one or two places where a few spicules of bone were observed surrounding a cellular structure which resembled bone marrow. The medulla was evidently hyperplastic and at one point was the area which at postmortem had been described as possibly an adenoma. This turned out to be a mass of myeloid tissue almost completely surrounded by well formed bone, and containing within it at more than one point several miliary tubercles. Further search in other sections brought to light occasional tubercles in the cortex of the adrenal, usually closely associated with the myeloid areas. In the cortex these structures measured 1×0.5 mm.; in the medulla, the one large mass of myeloid tissue measured 6×3 mm.

Careful study of these anomalous foci gave definite evidence that they were actually and typically bone marrow surrounded by bone, as the figures will show. There was a rich capillary network with endothelial lining between which were numbers of lymphocytes, leucocytes, myelocytes, and giant cells, beside developmental forms of red blood cells. The bone was lined with periosteum and endosteum.

The fact that the combination of tuberculosis of the adrenal associated with bone and myeloid tissue (tuberculous osteomyelitis of the adrenal) is rare, is less interesting than the reason why the bone appeared. I am of the belief that the fundamental lesion was a chronic tuberculous one, and that the bone and medulla formation was secondary to the irritation produced by the tubercle bacilli. It seems to me that the reaction of the tissue to the tuberculous irritation showed itself in hyperplasia of the supporting tissue with only slowly progressing necrosis and slight deposition of lime salts, a combination of circumstances which led to the proper conditions for functional change on the part of the connective tissue cells.

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A CARDIAC FORMULA FOR THE RELATIVE MEASUREMENTS OF THE VALVULAR ORIFICES*

By Robert A. Keilty, M.D., Philadelphia, Pa.

THE purpose of this presentation is to offer a working basis for the determination of cardiac valvular stenosis or insufficiency. In an autopsy technic the incisions for opening the heart vary to a wide extent, each individual operator having a method of choice. I am in the habit of opening the left ventricle first, exposing the aortic and mitral in order, then the right ventricle, exposing the tricusped and pulmonary orifices. Thus aortic, mitral, tricusped and pulmonary may be examined in order. Hereafter in this paper these openings are designated A. M. T. P.

The most accurate method of determining valvular stenosis or insufficiency is by means of measuring the various valve orifices after they have been incised. The normal diameters as given by different authors vary to some extent. For example, in centimeters, A 8.0, M 10.5, T 12.0, P 9.0, A. J. Smith; A 7.7, M. 10.4, T 12.0, P 8.9 and A 8.0, M 10.9, T 12.7, P 9.2, Mallory and Wright after Krause; female A 7.3, M 9.7, T 12.9, P 8.5, and male A 8.2, M 11.1, T 13.7, P 9.1, Shennon.

These measurements were always confusing to me until a few years ago when I noticed the following ratio was almost a constant factor, A 7, M 9, T 11, P 7, 7-9-11-7. These are the only figures one needs to remember. In the same proportion the orifices vary up to A 9, M 11, T 13, P 9, 9-11-13-9. Thus a heart 8.5-10.5-12.5-8.5 for example would be within normal proportions. In working such a formula the weight of the heart must be taken into consideration. In a small heart of 300 grms. we might expect to find 7-9-11-7, while in a large heart of 600 grms. 9-11-13-9. There may be said to be a variation within the normal, as applied to stenosis or insufficiency, of the following 7-9, 9-11, 11-13, 7-9.

It will be found that the measurements of the aortic and pulmonary valves are about the same, the pulmonary always a little larger. The mitral is about 2 cm. wider than the aortic and the tricusped about 2 cm. wider than the mitral.

In aortic stenosis, for example, with a large heart we might expect a valvular formula similar to this A 6, M 11, T 13, P 9, 6-11-13-9; in aortic insufficiency with a heart of the same weight A 11, M 11, T 13, P 9, 11-11-13-9.

In view of the great number of hearts, at least as found at autopsy, which show right-sided dilatation to some degree the measurement of the tricusped will vary more than of the other orifices. This variation in most instances will be found relative rather than organic and may be expressed in figures 7-9-14-7.

The figures which I have given are not exact, that is, they are not the result of the measurements of a thousand hearts but they are offered as a work-

*From the McManes Laboratory of Pathology of the University of Pennsylvania. Presented before the Pathological Society of Philadelphia, Jan. 27, 1916.

ing basis, easily remembered, for the determination either of stenosis or insufficiency at the time of an autopsy.

Conclusions.

1. In the following order A-M-T-P a cardiac valvular formula in centimeters 7-9-11-7 may be used as a practical working basis for the determination of stenosis or insufficiency.

2. This varies in accordance with the size of the heart within these limits 7-9, 9-11, 11-13, 7-9, and rarely goes above or below.

3. The variations affect all valves in proportion.

4. Upon finding any marked deviation in centimeters in one or two valves from the formula as given one may be justified in concluding either stenosis or insufficiency is present in a given case.

THE COMPRESSION SYNDROME OF THE CEREBROSPINAL FLUID

By G. W. Brock, Atlanta, Ill.

IN 1903 Froin reported three cases in which on withdrawal of the cerebrospinal fluid, the fluid was yellow in color, coagulated spontaneously, and contained numerous cellular elements, mostly leucocytes.

Since that time there have been some thirty-five cases or more reported in the literature.

The significance of this syndrome was first brought to my attention by Dr. Charles Louis Mix, and in the "Murphy Clinics" of April, 1915, he gives a summary of the cases reported up to that time, including one of Dr. Murphy's cases.

In the *Journal of the American Medical Association* for July 10, 1915, is an extract from the *Wiener klinische Wochenschrift*, of two tumor cases showing this syndrome reported by Schlesinger, one due to an extradural tumor, the other an angiosarcoma of the cauda equina.

The conditions leading to this syndrome may be any traumatic or pathologic process which cuts off the lower portion of the spinal canal, either completely or partially, forming a separate sac, the obstruction to the lumen of the canal usually being somewhere between the sixth thoracic and third lumbar vertebræ.

These conditions include: Trauma with hemorrhage in which the sleeve-like clot becomes organized; inflammatory reactions, with the formation of a fibrous tissue barrier, the inflammation being in the form of an acute meningitis, a pachymeningitis, or meningomyelitis, due to irritants injected from without, as stovaine in one case; or commonly bacterial in origin, including tuberculosis and syphilis.

Tumors or cysts of the conus terminalis or cauda equina or a Pott's disease with pressure, may be the etiologic factor.

Any one of the above lesions when occurring in the area of the lower segments of the cord or the cauda and their coverings, may produce this syndrome of xanthrochromia, spontaneous coagulation, and hematolymphocytosis.

The coagulation may be immediate, causing stoppage of the needle, or within a period of two or three hours, due to the amount of fibrin present in the exudate.

The cellular elements are most commonly entirely made up of lymphocytes.

Subsequent punctures may give a spinal fluid that varies in color from clear to a marked yellow; coagulation may be absent, when at some other puncture the fluid coagulates in the lumen of the needle; and the number of cells may vary from none to a numerous lymphocytosis.

This variance in the findings in the examination of the cerebrospinal fluid is due to the amount of walling-off in the inflammatory conditions, or the so-called "ball-valve" action, mentioned by Mix, as exerted by some tumors, which may permit the cerebrospinal fluid to pass the obstructing lesion, or make of the distal portion of the spinal canal a closed sac.

Sicard and Descomps were the first to recognize this meningeal pouch to be the essential physical factor in producing this change in the cerebrospinal fluid.

Three types of cases have been described: First, Acute cases of meningitis, some presenting the syndrome of a Landry's paralysis; Second, Paraplegia either spastic or flaccid, depending on the level affected, most of the cases being due to a pachymeningomyelitis of the conus terminalis. The symptoms, besides those of paralysis, are variable, disturbance of sensation and sphincteric control, with severe pain being most constant; Third, Tumors and cysts, either of the conus terminalis, cauda equina or the dura.

The duration of the symptoms leading to the production of this syndrome are reported as from a few days to seventeen years, although in long-standing cases it would be impossible to say how long this syndrome had been present.

Klienberger has reported three cases, two due to tumor, and the third as probably due to dementia paralytica, the etiology of the following case:

CASE REPORT.—M. L. P., male, age 34. Admitted to the Peoria State Hospital, Oct. 17, 1913, and assigned to the service of Dr. J. K. Pollock, physician in charge of the male receiving service.

Occupation, farmer; civil condition, married. The attack was of one year duration. No history of alcohol.

Physical Examination.—Well built, weight 160 pounds; pressure sore on right scapula and right knee, marked tremor, loss of sphincteric control. Gait very unsteady, with feet far apart. Pupils unequal and irregular, but reacted to light and accommodation. Deep reflexes exaggerated, the left knee more so than the right; Gordon and Babinski absent.

Mental Examination.—Disoriented for time and place, judgment extremely deficient, some grandiose ideas concerning wealth. Diagnosis, Dementia paralytica of the dementing type.

Three days after admission a lumbar puncture was made, which gave the following findings: Amount of cerebrospinal fluid withdrawn 15 c.c.; color lemon-yellow; Noguchi and Nonne-Apelt tests both positive; Lange's gold solu-

tion reduced to a white color in dilutions of 1-20, 1-40, and 1-80; cell count 50 cells per cm.; lymphocytes.

After about one hour the fluid coagulated in the test-tube into an almost solid mass. Wassermann strongly positive. Autopsy denied.

THE PRESENT STATUS OF THE ADRENAL PROBLEM*

By R. G. Hoskins, Ph.D., Chicago, Ill.

TO attempt to formulate a conception of the significance of the adrenal glands in the existing state of the literature is a somewhat precarious undertaking. This literature although extensive is by no means consistent nor complete. One can find recorded data that support almost any possible conclusion. His final conception rests therefore, to a considerable extent, on his selection and evaluation of more or less conflicting evidence.

From the confused maze, however, a few facts of major importance stand out unquestioned. Before taking up the present status of the adrenal problem to afford a background these may be briefly reviewed. Although the adrenals had long been recognized as definite anatomical structures, the first significant contribution to our knowledge of their function was made by Addison, an English clinician. In 1855 he reported that adrenal deficiency gives rise to a characteristic syndrome the cardinal features of which are muscular weakness, circulatory depression, gastrointestinal disturbances and deposition of a dark pigment in the skin and mucous membranes.[1] This discovery constitutes the most important evidence as to adrenal functioning even as we know it today. It also raises the most insistent question that still remains to be answered, namely,—What is the specific explanation of this complex; Why does an animal deprived of its suprarenal tissue inevitably die?

The next significant discovery was made by Oliver, another clinician. In an investigation of the effects of various gland extracts on his patients he discovered that suprarenal material is especially potent. In collaboration with the Edinburgh physiologist, Schaefer, he then undertook a systematic investigation of the properties of suprarenal extracts. It was discovered among other things that such extracts have a remarkable stimulating effect upon the circulatory apparatus, causing great augmentation of blood pressure. These observations were published in 1894-5.[2] The discovery was made independently by Szymonowicz, however, and published in 1896.[3]

The isolation of the so-called "active principle"—better *an* active principle—of the extract was soon after accomplished. Abel in 1897 secured it as a benzoyl addition product which he called "epinephrin."[4] Takamine and Aldrich in 1901 succeeded in isolating it as a pure crystalline substance.[5] They called it "adrenalin" which term was adopted as a trade name by the

*From the Laboratory of Physiology of the Northwestern University Medical School. Read before The Institute of Medicine of Chicago, January 28, 1916.

pharmaceutical firm by which they were employed. As a matter of usage adrenin seems now to be the favorite term for the substance. It belongs chemically among the benzene ring compounds, as is indicated by its name dioxyphenyl-ethanol-methyl-amine. Its composition suggests that it may be derived from tyrosin, one of the amino acids obtained in protein digestion. It is formed in the medulla but not in the cortex of the suprarenal.

The next outstanding discovery was made by a group of English investigators,—particularly Elliott.[6] His elaborate paper of 1905 has become a classic. He showed conclusively that adrenal extract stimulates selectively the neuro-cellular endings of the sympathetic nervous system wherever these are found. The effect—at least of physiologic quantities—depends in any organ particularly upon three factors: If the organ receives no sympathetic fibers no effect is produced. If the sympathetic fibers are relatively little used in every day life the effect of adrenin is correspondingly restricted. The effect is either augmentation or depression of the function of a given tissue depending upon whether its sympathetic fibers are excitatory or inhibitory. Thus, the uterus is stimulated whereas the intestine is depressed. This discovery seemed to many largely to settle the adrenal problem. But to this point we shall revert.

Another recent discovery that will probably be recognized as of major importance is that the adrenals are particularly influenced by emergency conditions. A conception of the "emergency function" of the glands based largely upon work done in his laboratory has recently been formulated by Cannon.[7] Various aspects of this function have been worked out and published in a series of joint papers since 1911. Cannon in studying the activities of the alimentary tract had noticed that an outburst of emotion, either fear or anger, resulted in a checking of peristalsis. This might well be due to an outflow of impulses throughout the sympathetic nervous system,—a phenomenon that is well known to occur under such circumstances. However, the investigator was struck by the fact that the depression of peristalsis persisted for some time after all signs of emotion had subsided. Adrenin was known to inhibit peristalsis. The adrenal glands were known to receive sympathetic fibers and hence supposedly to receive a share of the sympathetic impulses. It was suspected then that the prolonged alimentary depression might be due to an after discharge of adrenin from the excited glands. Direct research confirmed the suspicion.

Cannon in collaboration with de la Paz first studied the effect of violent emotions upon the adrenin content of the blood.[8] The method used was briefly this: A cat was bound to a comfortable holder. Then under local anesthesia a femoral vein was exposed and opened. This permitted the introduction of a long flexible catheter into the vena cava so as to secure a sample of blood from the level of the adrenal veins. The animal then had its sensibilities harrowed by the threatening presence of a barking dog. When the angry passions of the cat were sufficiently aroused a second sample of blood was secured. The two samples were immediately compared as to adrenin content. To find a suitable test object that could be used with defibrinated blood was for some time a baffling problem. Finally, however,

strips of longitudinal muscle of the cat's intestine were found to be suitable. These immersed in normal blood will undergo spontaneous, long continued, rhythmic contractions. If a trace of adrenin is added, however, the contractions immediately cease. By means of this test the two samples of blood were compared. That obtained while the animal was quiet showed no detectable adrenin. That, however, from the excited animal caused a marked depression of the intestinal strip. By suitable means it was shown that the depressing substance actually was derived from the adrenal glands. It was concluded, therefore, that during times of emotional stress the adrenal glands are stimulated to pour into the blood stream enough adrenin to exert a significant influence.

The problem then presented itself: Is this adrenin discharge purely a psychic phenomenon? In other words, could it be produced in an unconscious subject? That problem was investigated by Cannon and Hoskins.[9] Since pain is the most common source of emotion its effect on the adrenals was investigated. It was not advisable to subject the experimental animals to actual pain, however, hence its physiologic equivalent, sensory stimulation under anesthesia was employed. A stimulating electrode was adjusted to the sciatic nerve. Then a sample of blood was secured by the method previously described. A strong tetanizing current was applied to the nerve and after a short period of stimulation a second sample of blood secured. In the meanwhile it had been found that segments of rabbit intestine are more sensitive and more easily secured than are intestinal strips.[10] In this and subsequent researches along this line, therefore, such segments were used for the tests. It was found just as in the earlier experiments that blood secured after stimulation of the sciatic gave a clean-cut adrenin reaction. These results have been confirmed by other investigators, notably Elliott,[11] who has observed that sensory irritation results in exhausting the adrenin content of the gland. This fact has an obvious bearing on clinical practice. Any severe surgical operation is essentially a repetition of the experiment described. Adrenal exhaustion, therefore, is one of the immediate results of any such operation. Similarly, it was found that asphyxia causes adrenal discharge,— a fact that has also been confirmed in various ways.

Since this adrenal discharge may be purely reflex, and since reflexes are usually of direct benefit to the individual, the question arises: Of what use is the arrangement? McDougal has suggested that emotions are the mental representation of bodily activities: anger represents combat, fear represents flight. In case combat results, pain is experienced. Either flight or combat causes partial asphyxia, that is, breathlessness—at least until the body has had time to adjust to the conditions. All of these cause adrenal discharge. The adrenal discharge might be supposed, therefore, to aid in some way in strenuous muscular activity; that is, to integrate the body for muscular response to the stressful condition which caused the discharge.

As a matter of fact, the injection of adrenin does have a number of effects that aid in violent muscular activity. A quiescence of the whole digestive system results and the blood is shifted from the splanchnic circulation to the active muscles, and to the nervous and respiratory organs. The cir-

culation in these organs is further increased by a rise of blood pressure resulting from vasoconstriction of the splanchnic area and often of the skin, as well as by augmented cardiac discharge. In a direct investigation Hartman[12] working in Cannon's laboratory has recently shown that a given dose of adrenin simultaneously dilates the vessels of the muscles and constricts those of the splanchnic organs. A discharge of dextrose occurs, leading to hyperglycemia, whereby the laboring muscles obtain a better supply of energy material. This hyperglycemia has been observed as a direct result of strong emotion as well as of artificially augmented adrenin. The well-known bronchodilator effect of adrenin is of obvious utility in aiding free breathing whereby the oxygen necessary to violent activity is secured. Most striking of all, however, the laboring muscle itself is directly benefited. Cannon and Nice[13] observed that after the injection of adrenin or stimulation of the splanchnic nerves to cause adrenal discharge, the efficiency of a fatigued muscle is improved, sometimes 100 per cent. Gruber[14] has found that recovery from fatigue is greatly hastened by the injection of adrenin. The explanation of this fact is not definitely known, but a hint is offered by Evans and Ogawa's observation[15] that in the heart at least the assimilation of oxygen is increased by this substance. Hartman's observation that adrenin has a selective vasodilator influence in muscle also comes to mind. Cannon, Gray and Mendenhall[16] have noted another phenomenon which is of further use to the individual in case the stressful condition results in bodily injury: Adrenin lessens the coagulation time of the blood. This is true, however, only within physiologic limits,—for such quantities as can be obtained by stimulating the splanchnic nerves. In case greater quantities are employed, the coagulation is interfered with. With proper regard to this fact, adrenin might well come into frequent clinical use in combating hemorrhage. Cannon suggests that the pains of labor are of direct use to the mother in causing adrenal discharge and thereby protecting her from post-partum hemorrhage. One thinks also of the stimulating effect of adrenin on the uterus. On the whole it seems to be established that in times of stress the adrenal glands are stimulated to discharge a secretion that is of use in integrating the body to cope with emergencies.

The question may now be raised: What part do the adrenals play during periods of more placid existence? A few years ago a very satisfactory theory could be constructed about as follows: An animal deprived of its adrenal glands dies. The blood pressure falls indicating that the sympathetic nervous system has failed in its function. Injection of adrenal extract causes a marked rise of pressure; therefore, the function of the adrenal glands is to maintain the tonus of the sympathetic nervous system. This might be designated the "tonus theory." Although several facts are in contradiction to it the theory still has considerable currency.[17]

The first question that arises when one considers the theory is this: Is there present in the blood stream as it leaves the heart enough adrenin to exert any appreciable influence on the sympathetic nervous system? In the earlier literature occur various statements which indicate that the blood does contain considerable quantities. The concentration is given as, for example,

one part in 10,000,000. But it is interesting to note that as the technic of such determinations has improved the dilution of adrenin has continuously approached infinity. The best method now known for such tests is to run the suspected blood through the capillary bed of a frog's legs and note the rate of outflow. Trendelenburg, the recognized authority on this method, has recently reported that the concentration of adrenin in arterial blood is at most not more than one part in one or two billions or only one-fifth enough to exert any appreciable effect on the sympathetic system of a mammal.[18]

The tonus theory can be considered from another point of view. It assumes that the adrenals are constantly supplying enough secretion to stimulate the pressor mechanisms. This being true the addition of a little more of the drug by vein should augment this pressor influence and send the tension higher. As a matter of fact quite the opposite occurs. Such injections characteristically lower the pressure. This observation was made by earlier investigators, notably by Moore and Purinton,[19] but was ascribed to impurities in the material used. In 1912 Hoskins and McClure[20] reinvestigated the matter using a supposedly pure drug. Their experiments were made on dogs. The depressor effect was easily demonstrated. The fact was confirmed for cats by Cannon and Lyman.[21] In 1913 Hoskins and McPeck[22] showed that light massage of the suprarenals leads to a similar fall of pressure.

Again,—if the adrenals are pouring into the blood stream enough secretion to hold up pressure sudden occlusion of the adrenal veins should cause a prompt fall of pressure. As a matter of fact such procedure leaves the pressure exactly where it was before.[23] It is a matter of hours before a fall occurs whereas circulating adrenin is destroyed within a minute or two.

Hoskins and McClure[24] have tested the theory in another way. If adrenin be injected into a vein at very small but gradually increasing rates at first no effect at all is to be seen. Then various changes occur. One of the earliest is depression of intestinal peristalsis. If simultaneous tracings of peristalsis and blood pressure are taken it is found that the gut is paralyzed before any rise of blood pressure occurs. One need not point out the futility of an arrangement that could maintain blood pressure only at the expense of gastrointestinal paralysis. Other evidence of similar tenor could be offered but this may suffice. Attractive as the tonus theory was it is no longer tenable.

But the fact remains,—adrenal extirpation is fatal and the final symptoms include a failure of functions that are under sympathetic control. Elliott[25] has offered the interesting suggestion that a minute quantity of circulating adrenin is necessary, not to stimulate the sympathetic system but to maintain its irritability. That is, the terminal neurocellular substance of the sympathetic system in the absence of adrenin is no longer able to transmit impulses. This possibility also was investigated in our laboratory. It seemed to us that animals at the point of death, such as Elliott worked on, are not capable to giving any very significant information. All sorts of secondary factors may enter into the experiment. If sympathetic failure is a characteristic feature of the syndrome, it should appear at an early stage. It was found that at a time when the animal deprived of its adrenals is showing marked evidence of that fact,—when it can scarcely sustain its own weight,—its vasomotor system responds to stimulation

perfectly well.[26] Vasomotor—i.e., sympathetic failure, therefore, is to be regarded as a secondary feature. Both muscular and cardiac weakness precede it.

It would seem probable that if a trace of adrenin is essential for sympathetic functioning, the vasomotor reactions should be improved if an animal previously deprived of its adrenals were to receive a continuous injection of very dilute adrenin for, say half an hour. In carrying out this experiment the surprising fact was noted that such injections often actually impede sympathetic functioning. In some cases pronounced block was demonstrated.[27] This observation, which was confirmed many times both in normal animals and those deprived of their adrenals, seems to dispose of the alternative theory that adrenin facilitates sympathetic functioning. The conclusion follows then that the remarkable effect of larger doses of adrenin on the sympathetic system is of use in emergencies only.

The possibility still remains that minute quantities of adrenin are necessary for the metabolism of other tissues, for example, as Crile supposes, the brain cells.[28] If such were the case,—if adrenin failure were the significant feature in adrenal deficiency—it should be possible by continuous injections of adrenin to preserve the life of the operated animal. As a matter of fact no significant prolongation of life can thus be achieved.[29] Laying all theories aside and facing this fact, one can scarcely escape the conclusion that adrenin has no essential connection with the Addison syndrome or its laboratory equivalent,—that it is merely a reserve resource for use in emergencies.

The cause of death then in adrenal deficiency remains still an unsolved riddle. We are led back to the theories that prevailed before the potency of adrenal extracts was discovered. Do these glands perchance have a detoxicating function? Is the essential feature in adrenal death a toxemia? If so, the toxin should be detectable,—particularly since it is often potent enough to kill a dog within a few hours. There are on record some early observations which indicate that such a toxin can be demonstrated. Abelous and Langlois[30] reported that the blood of guinea-pigs dying after adrenal extirpation is fatal to frogs. In view of the possibility of the formation of various decomposition products, however, the burden of proof is on the experimenter to show that the toxin is specifically due to adrenal failure. Negative results in such experiments are more significant than positive. The matter has recently been under investigation in our laboratory. The work is not entirely finished but enough has been done to indicate that the blood of dogs that have just died of adrenal deficiency is in no degree toxic to frogs. Other observers have failed to detect a toxin by transfusion experiments.

We are finally thrown back then to the vague conception of a failure of tissue metabolism as the most probable cause of death. The tissues that first exhibit weakness are the cardiac and muscular. The most satisfactory theory for the time being, therefore, would seem to be that the adrenals contribute to the blood stream some unknown substance necessary to the metabolic processes of these active tissues. The best evidence indicates that this hypothetical substance is derived, not from the adrenal medulla which supplies adrenin, but from the cortex of the gland. At any rate in animals in which the two parts of the gland

are separate, death follows when the cortex homologue is removed leaving the chromaffin tissue unharmed.[31]

One other line of evidence bears on the question as to the function of the cortex. In various sorts of sex-gland anomalies hypertrophy of this tissue is observed. Most striking, perhaps, is the fact that sexual precocity in children has been shown to be accompanied by marked adrenal hypertrophy or hypernephromata.[32] In some experiments recently made[33] evidence has been secured that feeding suprarenal substance to young animals leads to hypertrophy of the testes. Further experiments along this line may lead to significant results.

In conclusion it may be said that the fundamental question remains yet to be answered: Why does the removal of the adrenal glands cause death? The trend of the evidence now available suggests that muscular metabolism is at fault. If that be true the solution, like that of many other of the most puzzling medical problems, rests in the hands of the biological chemists.

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LABORATORY METHODS

The Results of Quantitative Albumin Determinations from 6000 Cases of Suspected Tuberculosis*

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DURING the last five years considerable attention has been given to the determination of albumin in sputum by both qualitative and quantitative methods and numerous contributions to the literature on this subject have been made by several writers. A review of this literature is somewhat interesting but to one without extensive personal experience such review would seem rather confusing. The summary of conclusions drawn by different writers varies as to the value of albumin findings from an opinion that the determination has no practical value in the study of respiratory diseases to an opinion that it is one of our most valuable aids in the diagnosis of respiratory affections. There appear to be just reasons for these varying conclusions based upon several factors. Some of the publications are the result of study from a number of cases entirely too small to justify any conclusion whatsoever. Some writers appear to have been biased in their opinion either before their work was begun or by their early results and have thus failed to give logical consideration to their findings as a whole. Most important, however, is probably the general lack of uniformity in methods pursued and the personal equation influencing the delicacy of the reactions or determinations to be noted. Thus one writer reports albumin practically always present in sputum, regardless of the disease and another found albumin distinctly present in only a little over 60 per cent of cases of open tuberculosis, doubtful in 27 per cent, and entirely absent in 9.4 per cent of tuberculosis patients.

It must be conceded that almost all sputa and even normal saliva contains albumin-like bodies that can be detected or actually measured by means of certain well-known chemical processes. It is likewise true that after careful separation of mucin many sputa, even though containing distinct quantities of albumin, may fail to give certain of the albumin reactions especially to the less experienced observers.

Some time ago we† published a method for the quantitative determination of albumin in sputum with potassium ferrocyanid which is essentially as follows: A quantity of sputum, not less than 10 c.c., is collected in a dry receptacle in the usual way. (Samples containing blood should be rejected, as blood invariably contains albumin.) After smears for microscopic examination have

*From the Lansing (Mich.) Clinical Laboratory and the Upper Peninsula Hygienic Laboratory.
†Jour. Am. Med. Assn., lxii, 20.
Jour. Michigan Med. Soc., April, 1914.

been made a quantity of sputum not exceeding 10 c.c. is poured into a 50 c.c. graduated glass-stoppered cylinder. To this is added three times its volume of water containing 1 per cent acetic acid, diluting the sputum to 25 per cent. After vigorous shaking with the stopper in place, the mixture is filtered through filter paper directly into a graduated centrifuge tube and 10 c.c. collected. To this is added 5 c.c. of 5 per cent solution of potassium ferrocyanid in water and the tube whirled in a centrifuge for five minutes at average speed. The amount of albumin is most conveniently recorded in volume per cent, each 0.1 c.c. on the tube being 4 per cent by volume after correcting for the original dilution. Absolute accuracy is impracticable in this work, so only approximate results should be recorded. If desired, the volume per cent may be calculated to weight per cent according to the method of Purdy for urinalysis.

We have since the first publication employed this procedure on over 6,000 sputa and it has proved entirely satisfactory. Various modifications that have been proposed by others have not been found satisfactory by us.

The 6,000 cases offered here are taken in consecutive order from sputa received from general practitioners in Michigan and represent what the average physician submits from what he considers suspected cases of tuberculosis. Special examination from selected cases are not included in the table. For the purpose of convenience we have divided these cases into two groups, namely, those containing tubercle bacilli and those not containing tubercle bacilli. Out of the total of 6,000 sputa examined, tubercle bacilli were present in 1,412 and absent in 4,588. The albumin determinations gave results as follows:

PRESENT	TUBERCLE BACILLI	ABSENT
1,412	Sputa Examined	4,588
3%	Albumin Absent	40.0%
97%	Albumin Present	60.0%
	Quantitative Determination	
5.0%	Below ½% by volume	14.4%
3.0%	Between ½-2% by volume	4.0%
7.0%	" 2-5% " "	8.0%
10.0%	" 5-10% " "	9.2%
36.0%	" 10-25% " "	15.4%
26.0%	" 25-50% " "	7.8%
10.0%	Above 50% " "	1.2%

In addition we have examined discharges from a considerable number of abscesses as well as secretions and exudates from various other parts of the body. Such abscesses have generally shown albumin ranging in volume from 10 per cent to 35 per cent. Two cases of pulmonary edema gave albumin over 50 per cent by volume. Twenty-eight cases of pneumonia in various stages gave albumin ranging from 10 per cent to 50 per cent by volume. Eleven cases of pleuritic effusion, diagnosed as tuberculosis gave albumin above 50 per cent by volume.

One case of tuberculosis of the bowel gave about 1 per cent volume of albumin in the stool and seven cases of tuberculosis of the kidney showed from 10 to 25 per cent of albumin by volume in the urine.

Our experience indicates that normal saliva, urine, feces and stomach con-

tents, following an Ewald test meal, do not show albumin by the method we have employed.

In urine a negative albumin reaction appears to us to preclude active tuberculosis of the kidney and we believe albumin in the urine is constantly associated with active tuberculosis in any part of the urinary tract. The same is probably true of the bowel. Several writers state that the finding of tubercle bacilli in the stool is conclusive evidence of intestinal tuberculosis. This appears to us to be an error as we have been able to show that tubercle bacilli can be demonstrated, with sufficient search, in the stool of practically every case of open pulmonary tuberculosis. Some tubercle bacilli are inevitably swallowed and pass through the alimentary tract. Soluble albumins swallowed do not normally appear in the stool and the appearance of such albumin in the stool generally indicates that an albuminous exudate is entering the lumen of the intestine lower down.

In dealing with sputa the interpretation of results is somewhat difficult. The composition of what is ordinarily termed a sputa may be rather complex, including saliva, discharges from the nose, and occasionally the accessory sinuses, throat, lungs and even vomitus. Rigid interpretation of the findings should seldom be made unless the interpreter is fairly certain that the material examined comes from the area in question. The presence of tubercle bacilli in a sputum is not in itself sufficient to indicate that we are dealing with a pulmonary sputum.

We have been able to show from cases of open pulmonary tuberculosis that the bacilli may be found in saliva, in water used for gargling the throat and even water used for rinsing the mouth. Such material would obviously be relatively free from albumin. Further, tuberculosis in a certain portion of a lung does not exclude other pathological conditions in different portions of the respiratory passages. One patient actually furnished in the same day a sputum free from tubercle bacilli and albumin and another containing a fairly large number of tubercle bacilli and 8 per cent albumin. Such conditions are probably rare, but we believe the possibilities they present offer satisfactory explanation for the low or absent albumin findings in exception to the general rule.

It cannot be said that there is any specific relation between tuberculosis and the presence of albumin in sputum, for albumin appears to be constantly present in pneumonia, pulmonary edema, and abscess of the lung as in abscesses of other parts of the body. Our findings indicate that albumin is present in the sputum of practically all cases of active pulmonary tuberculosis and that in over 80 per cent of these the amount is above 10 per cent by volume. In chronic rhinitis, pharyngitis, laryngitis, bronchitis and asthma albumin is generally absent or present only in traces. In acute conditions the findings are less constant, while the absence of albumin is the rule in all superficial inflammations of mucous membranes, abrasions or lacerations of the surface epithelium are occasional accompaniments to such cases and give rise to serous or bloody exudates that contain albumin in abundance. An absence of albumin we believe excludes active tuberculosis as the source of that particular sputum.

The presence of albumin *per se* therefore has no great diagnostic or prognostic value and it is only when quantitative determinations are considered together with other findings that the most valuable conclusions may be drawn.

It has been generally accepted that active tuberculosis in the various parts of the body gives rise to a lymphatic exudate and it has been customary among laboratory men to diagnose tuberculosis from effusions high in lymphocytes when coming from sources outside of the lung. The same principles, however, do not appear to have been generally applied to the study of sputa.

We believe that a predominance of lymphocytes together with high albumin justifies the diagnosis of tuberculosis in the absence of tubercle bacilli from a sputum exactly as well as do the same findings from a pleuritic exudate or spinal fluid. Either albumin or lymphocytosis, however, taken alone is unreliable and does not justify such a diagnosis.

The most valuable factor of the albuminous exudate as it occurs in active pulmonary tuberculosis is the fact that it is independent of the presence or absence of tubercle bacilli in the sputum. Consequently it is independent of whether the lesion is open or closed. We have therefore in the albumin determination an index to the activity of a lesion that appears before the tubercle bacilli are found and generally before secondary infection has taken place. Under such conditions a high albumin associated with a predominance of lymphocytes appears frequently and early and gives us a reliable basis for diagnosis. As a means of prognosis the quantity of albumin appears to be a direct index to the activity of the lesion. We have followed a considerable number of cases through their incipiency and have had the good fortune also to follow some cases well into recovery. The findings on such cases indicate that albumin appears early before tubercle bacilli are present, diminishing and disappearing with the arrest of the process.

A Clinical Method for the Determination of Carbon Dioxide in Alveolar Air

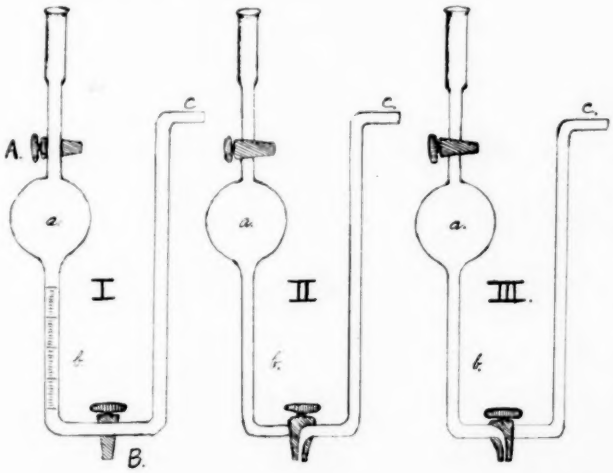
By J. J. R. Macleod, M.B., Cleveland, Ohio.

THE percentage of CO_2 in the alveolar air corresponds to the tension of this gas in the blood, that is, to the amount of CO_2 which is in simple solution in the plasma (Haldane and Priestley, Krogh). Since this tension of CO_2 in the blood depends on the equilibrium between acids and bases therein contained, it will become less whenever an excessive amount of acids other than CO_2 is present, just as would be the case if we were to add some strong acid to a solution of bicarbonate. In both cases, after the immediate displacement of CO_2 by the stronger acid, a smaller amount of bicarbonate, and therefore of free CO_2, would remain in the solution. When a subnormal percentage of CO_2 is present in the alveolar air, therefore, it indicates that there is an excess of fixed acids in the blood. *It indicates that a severe degree of acidosis exists,* and is a danger signal of impending coma. It has been known for long that very low alveolar CO_2 percentages are obtained in late stages of diabetes, but until recently the fact could not well be used for diagnostic purposes because of the

more or less difficult method (of Haldane) which had to be employed for the estimations. Fridericia[1] has recently furnished us with a sufficiently accurate and yet very simple method, which should now make estimations practicable in the clinic.

This method is as follows: The necessary apparatus consists of a U-shaped glass tube (see Fig. 1) with stopcocks A and B, as depicted in the accompanying figures. A is a simple stopcock, but B is three-way, as shown in Figs. II and III. Between these stopcocks the tube has a bulb blown on it so that the total capacity between A and B is exactly 100 c.c., the part of the tube below the bulb being graduated 1/10 cubic centimeters. A cylindrical vessel large enough to hold the apparatus and containing water at room temperature, an ordinary evaporating dish, a 20 per cent solution of KOH, a saturated solution of boric acid, and a rubber bulb fitting to the side tube C, are also required.

To make an estimation the apparatus is first of all rinsed out with the boric



acid solution (which absorbs practically no CO_2). The patient (who may be lying in bed) is told to take the end of the tube, C, in his mouth after an ordinary expiration and to blow out all the air he can through the apparatus, with A and B open as in Fig. 1. When he has done this and before removing the apparatus from the mouth, the tap A is closed. The entire space in the apparatus is now filled with air which must have come from the alveoli, since the "dead space" of the lungs (i. e., the mouth, trachea and bronchi) has a capacity of not more than 130 c.c., whilst the volume of air, which even a weakened patient could blow out, measures at least 1,200-1,500 c.c. The apparatus is now placed for 5 minutes in the clyinder of water so as to cool it down to the temperature of the room, after which it is removed and the tap B turned into the position indicated in Fig. II. Between A and B we now have 100 c.c. of alveolar air at room temperature. The wider portion above stopcock A should alone be handled in removing the apparatus, so as not to warm the gas in the confined portion. (During the cooling process some of the air in the part of the tube

[1]Fridericia, L. S.: Eine klinische Methode zur Bestimmung der Kohlensaurespannung in der Lungenluft, Berl. klin. Wchnschr., 1914, li, p. 1268.

between B and C will have been sucked into the graduated portion because of shrinkage, but this will not vitiate the analysis, because it also is alveolar air.)

It is now necessary to ascertain what proportion of this 100 c.c. of air is CO_2. This is done as follows: Turn the stopcock B into the position in Fig. II, place the side tube of the stopcock in an evaporating dish containing 20 per cent KOH, and by means of the rubber bulb suck this solution up to the bend of the tube C. Turn stopcock B so that it is completely closed, and after carefully removing the rubber bulb from C, again turn stopcock B as in Fig. I, (but with A still closed) so that some KOH solution runs into the graduated portion of the apparatus. In doing this care must of course be taken that no air enters the graduated portion. Stopcock B is now turned as in Fig. II and the KOH solution in the free tube allowed to run out, after which this stopcock is turned off and the apparatus inverted several times so that the KOH may absorb all of the CO_2 in the confined air. The apparatus is now submersed in the cylinder of water up to stopcock A and B is turned, as in Fig. II, so that water can run into the graduated tube. It will run in because a partial vacuum has been created on account of the absorption of CO_2. After allowing time for temperature conditions to become equalized (5 minutes), the apparatus is now cautiously raised until the water inside and outside the graduated tube stands on the same level. Stopcock B is turned off and the apparatus removed and the graduation read at which the meniscus stands. The reading gives us directly the percentage of CO_2 in the alveolar air.

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EDITORIALS

The Role of Fixed Tissue Cells in Protecting the Body Against Infection

IT will be remembered that the science of immunity, after being founded on the basic observations of Pasteur on active immunization, developed along two parallel lines,—one the investigation of the direct action of serum constituents on the invading bacteria and their products, the other centering upon the activities of phagocytic cells. These two schools have more recently met upon common ground in the recognition of cooperative activity between plasma and cells. Though it is generally not recognized, the idea that the serum antibodies might act upon the bacteria preparing them for phagocytosis was expressed by Metchnikoff himself early in his investigations.[1] However the firm establishment of this fact was postponed until its experimental proof by Denys and his collaborators, by Wright and by Neufeld. Since its recognition there has been a general tendency to look upon phagocytic immunity as the perfect mechanism for protection and there has been a suspicion that the reaction of serum antibodies and bacteria without the cooperation of phagocytic cells might even result in injury rather than defense by the liberation in such a process of toxic substances from the bacterial protein, substances analogous at least to those obtained by hydrolytic cleavage by Vaughan. In infections with such organisms as the cholera spirillum and the typhoid bacillus, it has been shown by Bordet, and more recently by Gay and Claypole, that immunized animals responded to

reinjection with a leucocytosis far in excess of that following the first injection into normal animals.

Although, therefore, the phagocytic functions of the mobile leucocytes have been very intensively studied, yet insufficient attention has been paid to the phagocytosis carried on by fixed tissue cells. We have had a vague understanding of such phagocytic processes in tuberculosis, leprosy, blastomycosis, rhinoscleroma, and possibly syphilis, but the difficulty of experimentation has been such that little progress has been made. It has been known that the cells of the liver and spleen, young connective tissues, the dust cells or alveolar[2] cells of the lung, and many other cellular elements not mobile in the circulation, could take up bacteria. It has been supposed that tubercle bacilli not digested within the polymorphonuclear leucocytes were destroyed by the giant cells appearing in tuberculous lesions, and the writer, with Cary, showed some years ago that rat leprosy bacilli not injured morphologically during prolonged sojourn within polymorphonuclear leucocytes were rapidly digested in spleen cells grown in vitro. Adami,[3] moreover, as early as 1899 called attention to the fact that, during normal life, living bacteria may enter the portal circulation to be very largely destroyed in the liver, the lymphatic organs or the kidneys, a condition of which he speaks as *sub-infection*. However all this work has established no definite relationship between fixed-cell phagocytosis and resistance.

In a recent paper, which appears to us very important in its general significance, Kyes[4] has demonstrated a remarkable parallelism between an instance of natural resistance and fixed-cell phagocytosis. He chose for his experiments the pneumococcus as the infecting agent and the pigeon as the naturally resistant animal. Apparently it is impossible to produce symptoms of disease in this animal with large doses of virulent pneumococci, and this has been hitherto regarded as largely dependent upon the high body temperature of pigeons. However Kyes's experiments seem to show that the temperature alone cannot explain the rapid disappearance of the bacteria when injected into this animal, since 96 hours' exposure at 43° C. will not destroy pneumococci, but, injected into the blood stream, several billions are taken up within ten minutes and are totally destroyed within 72 hours. Kyes's work does not imply, nor does he draw the conclusion, that the temperature has no protective value; in fact, it is not impossible that the complete and rapid destruction of the bacteria is made possible by the high temperature and the consequent failure of multiplication of those injected. However Kyes has discovered a mechanism of bacterial destruction which, though vaguely indicated in earlier work, has never before been so thoroughly recognized. He has found that pneumococci injected into the general blood stream in pigeons are rapidly localized in the liver and spleen and then are taken up within the hemophagic cells, which, localized both in the liver and spleen, are supposed normally to serve the function of red blood cell destruction. The rapid destruction of the pneumococci by these cells is shown definitely by Kyes's work to constitute a very important factor in freeing the animal body of the invaders.

This work is remarkably similar in tendency to the work of Bull[5] of the Rockefeller Institute on the removal of typhoid bacilli injected into normal rabbits, in which Bull has for the first time demonstrated a possible immuno-

logical function for agglutinins. It appears that the injected bacilli rapidly disappear from the circulating blood. They are intravascularly agglutinated and the clumped bacilli are taken up by polymorphonuclear leucocytes and other cells in the liver and the spleen. A similar destruction of pneumococci in the liver and spleen has been shown to occur by Berry and Melick,[6] after intraperitoneal injection.

These observations have fortified the rapidly growing impression that, in the removal of bacteria from animal bodies, the fixed tissue cells may play a much larger part than hitherto suspected. They are, furthermore, gradually strengthening our belief that very little actual importance is to be attached to the bactericidal processes in the serum, which, indeed, may play a very secondary part in protection.

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—*H. Z.*

The Epiphysis (The Pineal Gland)

THE epiphysis is formed at the caudal end of the diencephalic roof. It consists originally of a thin ependymal diverticulum between the posterior commissure and the hebenular commissure. Subsequently its walls are thickened and incorporate some of the adjacent vascular mesoderm to form the adult organ (Streeter[1]). The epiphysis is therefore essentially an epiblastic organ, a fact which makes it difficult to account for the further fact that the most frequently encountered tumors affecting it are teratomas.

The evidence which connects the pineal gland with disturbances of growth and function of the body is incomplete, and is even less satisfactory than that concerning the thymus.

It is probable that the inaccessibility of the pineal has been the reason for the fact that very little experimental work has been carried out in connection with this gland, and for the unsatisfactory results of experiments, and that so little is known of its possible function. Up to within very recent years all that has been suspected of it has been based almost alone upon clinical data. It is true that since 1910 several investigators have made attempts to gain experimental data, but the great difficulties of operative procedures were not surmounted until 1915 when Dandy perfected a technic which permitted a quick complete removal after a very brief operation. It is perhaps unfair to a gland to draw conclusions regarding its place in the economy when the operative procedure alone produces a death rate of 75 out of 95 animals (Exner and Boese), or, in another series, of 12 out of 15 (Sarteschi).

The clinical data which have suggested that pineal abnormalities are associated with sexual precocity and adipose overgrowth, are derived chiefly from

the cases of Heubner (Oestreich and Slawyk), Ogle, Machell, Fränkl-Hochwart, and Raymond and Claude, in which the pineal was the seat of a teratoma. The experimental data emanate from the feeding experiments of McCord, Dana, Berkeley, Goddard, Goddard and Cornell. The clinical data have come as a rule from cases in which the pineal has been involved in a tumor growth, and therefore they are unsatisfactory, because under such circumstances one does not know to what extent other neighboring organs are modified, directly or indirectly by the growth. It is an interesting fact that the tumors in such cases have been almost without exception teratomas.

Since Heubner's report of his case in which a teratoma of the pineal was found at autopsy by Oestreich and Slawyk,[2] the attention of clinicians was focused upon the possibility that cases showing sexual precocity with or without similarly pronounced adiposity, were the subjects of pineal lesions. Later Marburg[3] from a study of the cases reported in the literature and his own (about forty in all), scheduled his conception regarding hypopinealism, hyperpinealism and apinealism, by which he introduced what he believed to be pineal complexes, by clinical study of which he could say that hypopinealism was associated with sexual precocity, obesity with hyperpinealism, and cachexia with apinealism. This seemed to indicate that hypophyseal lesions and pineal lesions tended in opposite directions, for while Fröhlich's syndromes might result for underactivity of the pituitary, an indistinguishable syndrome could follow overactivity of the pineal.

Marburg believed that the pineal was a functionally active gland only during the early years of life, and that during this period, it inhibited the development of the sexual side of growth, and that with its gradual involution during youth, its inhibitions were also gradually lost, until at the period of maturity, the pineal no longer had a necessary part to play in the economy. It seemed possible, following this conception, that if, before involution was complete, the pineal was destroyed, sexual and somatic development would go on apace relieved of the normal pineal inhibitions. It should be remembered, as Dandy says, that the whole structure of Marburg's hypothesis was based upon clinical and anatomical studies only, and not upon experimental work.

In 1910, experimental attempts to extirpate the gland were undertaken by Exner and Boese[4] who were able to discover no changes after incomplete or complete removal. Other experimental work has contradicted these results (Sarteschi, Foà). However the operative experimental results are no more contradicting than those based upon treatment with gland extracts or feeding of the gland substance. In the operative examples, however, the value of the work is modified by the technical difficulties which were not surmounted.

No less contradictory than the results of operative experiments have been those of feeding. McCord[5] fed guinea-pigs, chickens and dogs with pineal materials and believed that this treatment produced precocious sexual changes, accompanied by an increase in weight, and also accentuation of the mental condition in the direction of precocity, and Dana and Berkeley[6] reported weight increases above the normal in guinea-pigs, rabbits and kittens. In fifty children who were given pineal injections, however, growth in height and weight was

less marked than in controls, but there was a certain mental effect which was analogous to that which McCord had recorded in his puppies.

Dandy[7] summarizes the results of experimental work up to the time of his own report as follows: "Adiposity may result by feeding pineal extract (McCord, Dana, and Berkeley) or by complete or partial removal of the pineal (Sarteschi). Sexual and somatic precocity may result from feeding pineal extracts (McCord, Dana, and Berkeley), or from partial or complete removal of the pineal body (Foà, Sarteschi), or nothing may result from its partial or complete destruction (Exner and Boese)."

It was with the object of shedding a little light into the shady pineal region that Dandy perfected and put into practice an operative procedure which is free from objection. Little trauma is produced upon the brain. Little or no hemorrhage results, and the pineal can be removed completely. He has made a series of studies upon young puppies, from ten days to three weeks old, and also in several adult dogs of both sexes. His results are striking and confirm those of Exner and Boese, in that no evidence was obtained of any somatic, sexual or mental effects in the direction of inferiority or superiority. The conclusion seems to be, and Dandy reaches it, that the pineal is not essential to life and seems to have no effect upon the well-being of animals.

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—*P. G. W.*

Some Recent Contributions to Our Knowledge Concerning the Metabolism of Fat

THE mechanical view of the absorption of fat by the intestinal epithelium has now been entirely replaced by the chemical view. According to this the fat is split by the agency of the "lipase" of the pancreatic juice in the upper levels of the intestine into fatty acid and glycerine. The fatty acid becomes combined either with alkali or with the bile salts. Unlike the fatty acids themselves the soap and the compound of bile salts with fatty acids are soluble in the water of the intestinal contents. The dissolved glycerine and fatty acid are separately absorbed into the intestinal epithelium, in the protoplasm of which they re-combine to form neutral fat. The neutral fat thus produced gradually finds its way from the epithelial cells to the central lacteals of the villi, and thence by way of the thoracic duct, into the blood stream.

A most important application of this view is that substances other than fat will not be absorbed by the intestinal epithelium, however like fat they may be in their physical properties (i. e., emulsify well with dilute alkali, dissolve in the fat solvents, melt at the body temperature). Petroleum emulsion, for example, according to physical and microscopic examination, may be in every re-

gard identical with an emulsion of neutral fat, and yet if a compound emulsion composed partly of neutral fat and partly of some petroleum be administered to an animal, it will be found that all of the neutral fat is absorbed, but none of the petroleum. This can be demonstrated either by examination of the feces or of the lymph flowing from the thoracic duct during the absorption of the emulsion. Experiments of this type were performed several years ago by Henriques and Hansen, but the results, as stated above, were challenged by Bradley, who claimed to find some of the petroleum absorbed. It has recently been shown by Bloor,[1, 2] however, that the former investigators were correct. Obviously such an experimental result once and for all disproves the mechanistic theory of fat absorption.

Not only petroleums, but fat-like substances which are incapable of being attacked by the lipase of pancreatic juice, also fail to be absorbed, for example, wool fat, which is a compound of cholesterol and fatty acid. Now, one characteristic of all these substances that are not absorbed is that, although physically, and in many regards chemically identical with fat, they fail to be reduced to a water-soluble condition in the intestine. They are soluble in fat and in fat-solvents, but they are incapable of saponification in the intestine.

It might be objected to the above conclusions that, although undetectable, there is really some essential physical difference between emulsified fat and emulsified hydrocarbon. In order entirely to prove the case for the chemical theory, it is necessary to feed a neutral fat possessing some characteristic that depends on the manner of union existing between the fatty acid and glycerine, and then to see whether it appears in an unchanged condition in the thoracic duct. If it does so, the fat must have been absorbed through the intestinal epithelium in an unbroken, unsaponified, condition, for it is unlikely, in the resynthesis which occurs in the intestinal epithelium, that the fatty acid molecules would re-combine with the glycerine molecules in exactly the same manner as before.

There are, however, but very few qualities of neutral fat, apart from those of the fatty acids which compose them, by which they can be characterized. The most likely one is that of optical activity. None of the ordinary fats is optically active, although from chemical considerations it is quite conceivable that they might become so. In order to obtain such a fat Bloor[4, 5] conducted numerous experiments with the esters of stearic acid. He prepared an optically active mannitan di-stearate, but found it to have a very high melting point and to be only half as digestible as the ordinary fats. Its absorption was too slow and unsatisfactory to make it suitable for the above purposes. He, therefore, proceeded to prepare the di-ester of isomannitan with lauric acid, and he found the resulting compounds to be as well-absorbed as ordinary fat, and yet to possess very marked dextro-rotatory power, which of course they lose on saponification. This fat seemed suitable therefore for testing the above question. In a series of experiments, Bloor fed insomannid-di-laurate and by examination of the neutral fat present in the chyle flowing from the thoracic duct, found no evidence of the dextro-rotatory fat. This result confirms previous work by Frank, who found that the ethyl esters of fatty acids are not absorbed unchanged. The results of both groups of workers emphasize the probability that readily

saponifiable fatty acid esters do not escape saponification under the favorable conditions of the normal intestine. In other words, had the fats been absorbed unchanged, as would be required by the mechanistic theory of fat absorption, they would have appeared in the chyle in optically active condition.

These most important conclusions lead us to inquire as to the reason for this change in fat during its absorption. It cannot be for the purpose of preventing the absorption of undesirable fatty substances, such as the petroleum hydrocarbons or the wool fats, because such substances are so rarely present in our food. It is most probable that the breakdown and resynthesis of neutral fat occurs for the same reason that similar processes occur during the absorption and assimilation of protein. It will be remembered that protein is entirely disintegrated in the intestine into its so-called building stones. These are absorbed separately into the blood, which carries them to the tissues, in which they become resynthesized to form the body protein. And so it appears to be in the case of fats. The process, in other words, permits of the rearrangement of fatty acid molecules, as a result of which the newly formed fat is more adaptable for use in the organism. It comes to be more like the characteristic fat of the animal. There may be another reason for the process. It will be remembered that lecithins, which constitute the most important of the fatty substances of the cell itself, are mixed glycerides, that is to say, are compounds containing a variety of fatty acids. The rearrangement of the molecule of neutral fat which occurs during absorption may be the first step in the transformation of fat into lecithin.

In order to throw further light on the question, Bloor has performed a number of interesting experiments in which he compared the chemical properties of fats before and after their absorption. The criteria which he took were melting point, iodine value and mean molecular weight; the melting point represents the solidity of the fat, and the iodine value, its degree of unsaturation, that is, the number of double links that exist in the fatty acid chain.* Bloor found that during absorption very considerable changes in these two characteristics occur. For example, when fat with high melting point and low iodine value was fed, the fat in the thoracic lymph was found to give fat of distinctly lower melting point and higher iodine value. When fat with a low melting point and high iodine value was fed, the reverse change occurred, for the melting point of the thoracic lymph fat was higher and the iodine value lower. These results in the first case could be explained as due to the addition of oleic acid to the fat during its synthesis in the intestinal epithelium, and in the second case to the addition of some saturated fatty acid. When a fat was fed consisting mainly of glyceride of saturated fatty acid, but having a low melting point, the addition of oleic acid was still found to occur, as judged from the iodine value. This leads us to conclude that the change which occurs is not merely in order that the melting point of the absorbed fat may be lowered, but also for some chemical reason. In a fourth series of experiments, a lowering of iodine value occurred after feeding with a fat, namely, cod-liver oil, which already contained a high percentage of glyceride of very unsaturated fatty acid.

*These estimations were really made on the isolated fatty acids.

Evidently then the intestine possesses the power of modifying the composition of fat during its absorption, and this modification is apparently of such a nature that it causes a change toward the production of a uniform chyle fat, presumably the characteristic of the animal body. It remains of interest in this connection to consider the source of the oleic acid or of the saturated acid required for this synthesis. Bloor quotes work by other observers to show that the changes are probably greater than could be produced by admixture of the absorbed fat with the fat present in the normal fasting chyle.

Important work has recently been done concerning the fat present in the blood. Normally the blood contains only a small percentage of fat, but after a fatty meal it may contain so large an amount that the fat actually rises to the surface of the blood like a cream. By means of the ultra-microscope, examination of the blood in the dark field after a rich meal reveals the presence of glancing particles, the so-called "fat-dust." These particles are most abundant about six hours after the meal has been taken, and they gradually disappear again by the twelfth hour. These facts have been known for some time, but it has been impossible, either on account of the large quantities of blood required for a chemical examination or because of the inability to estimate the amount of fat from the density of the "fat-dust," to follow with any great degree of accuracy the exact changes that take place in the fat of the blood. It is, therefore, a most important contribution that Bloor should have succeeded in elaborating a method by which the fat-content of the blood can be fairly accurately estimated, using only small quantities of blood. This permits of a continuous series of observations over a considerable period. In Bloor's method,[8] the fat is extracted from the blood by an alcohol-ether mixture with moderate heat. An aliquot portion of the filtrate is evaporated in the presence of sodium ethylate; this saponifies the fat. The residue consisting of soap is well washed and is then treated with hydrochloric acid so as to precipitate the fatty acid. The density of the precipitate thus produced is compared in an optical apparatus, called a nephelometer, with a standard solution of two milligrams of oleic acid, treated in the same way. The lecithin and cholesterol may also be estimated in the same blood extract.

For lecithin[9] the above extract of blood, after the removal of the alcohol and ether, is digested by heating with concentrated HNO_3 and H_2SO_4. This decomposes the lecithin, liberating the phosphorus, a solution of the resulting ash being rendered faintly alkaline to phenolphthalein and then slowly added to a silver nitrate solution. The density of the precipitate thus produced is compared in the nephelometer with a precipitate produced in the same amount of silver nitrate by adding to it a standard phosphoric acid solution.

For cholesterol an aliquot portion of the above extract is saponified with sodium ethylate and then saturated with choloroform; the chloroform extract is mixed with acetic anhydride and H_2SO_4 (con.) until the bluish color is fully developed (Liebermann reaction), the intensity of which is then compared in a colorimeter with that obtained by similar treatment from a standard cholesterol solution.

By the use of these methods Bloor has made a careful study of the varia-

tion in the fat content of the blood under normal conditions in dogs. He has found[6] that the percentage of fat is remarkably constant under normal conditions, which, he says, probably indicates that in man also this is the case. The examination of the blood after a fatty meal showed that the increase in fat content began in about an hour, and reached its maximum in about six. This increase was not found in animals in which the thoracic duct had been ligated. Although this result would seem to contradict the view which some have held, that part of the fat which cannot be accounted for in the thoracic duct lymph is absorbed by way of the portal vein, Bloor does not commit himself on this point, but merely states the result and calls attention to previous work by d'Errico to the effect that the fat content of the portal blood is always higher than that of the jugular.

Very interesting results were obtained following the intravenous injection of emulsions of oil, either the so-called casein emulsion or colloidal suspensions. Up to a dose of 0.4 gm. per kilogram of body weight—which by calculation would suffice to raise the fat content of the blood by 100 per cent—these caused no increase in fat content. In order to explain this disappearance of fat, it might be imagined that the injected fat particles formed emboli in the smaller capillaries. Against such a view however is the fact that the particles of fat in these emulsions were one-half to one-seventh the size of a red corpuscle. Although this argument is no doubt of some weight, it ought to be remembered that the physical condition of these fine fat globules is not the same as that of the red blood corpuscle. Their surface condition may be such that they readily agglutinate so as to form small masses which may stick at the branching of the smaller arterioles and capillaries. Bloor himself suggests that the injected fat may be stored, possibly in the liver, since the fat in this organ, as we shall see later, increases in similar experiments. When twice the above quantity of fat was fed in the form of egg-yolk fat, some of it persisted in the blood for several hours. This increase may have been due to the flooding of the temporary storehouse with fat, or, more probably, to a retarding influence which lecithin may have on fat assimilation. That lecithin itself persists in the blood for a long time after intravenous injection had previously been demonstrated by Nerking.

A careful study was also made of the blood-fat during fasting. No increase was found unless the animal, by special feeding, had been stuffed with excess of fat prior to the fasting period. Narcotics were found to produce an increase in blood-fat, but ether produced this increase during the narcosis, whereas morphine and chloroform did not do so until after recovery. The explanation given for the ether effect is that a mixture of blood and ether has higher solvent power for fat than blood alone. The explanation for the chloroform and morphine effects is that a certain amount of breakdown of the tissue cells, in which lipins are set free, supervenes upon the action of these narcotics. At the conclusion of this paper Bloor suggests that there may be two places in the body in which surplus fat is stored: (1) a temporary storehouse, from which the fat is readily taken up and liberated, but which is of limited capacity, and (2) a more permanent storehouse, into which the fat is slowly taken up, but the capacity of which is very much greater. The temporary storehouse may

be under the control of some quickly acting fat-regulating mechanism, like that of the glycogenic function of the liver.

Most suggestive further observations by the same worker have recently appeared.[7] By comparison of the fat acid, lecithin, and cholesterol content of blood during fat absorption, it has been found that there is a steady but very variable increase in fatty acid, accompanied by no variation in cholesterol, but with an increase in lecithin, which varies from 10-35 per cent, but does not run strictly parallel with the fatty acid increase. It is pointed out that this increase in lecithin may represent that part of the absorbed fat which is intended for immediate use in the tissues. The more or less independent increase in lecithin observed in these experiments is of significance in connection with the fact, which has recently been established, that in many pathological conditions of so-called lipemia the increase does not affect the fats of the blood but rather the lipoids (i. e., lecithin and cholesterin). In yet unpublished work communicated to the Biochemical Society in December last, Bloor has found that separate analysis of blood plasma and whole blood shows the increase of lecithin to be much more marked in the corpuscles than in the plasma, whereas the fatty acid increase is confined to the plasma.

Besides the temporary and permanent fat storehouses (the so-called fat depots), there are two other places in the animal body where fat exists, namely, the protoplasm of the tissue cells and the liver. The immediate function of the fat in each of these places is not the same. In the depots, such as the subcutaneous tissue and the mesentery, the fat exists purely for storage purposes; in the protoplasm of the tissue cells it exists for the purpose of being oxidized to liberate the stored energy; and in the liver it appears to exist for the purpose of being prepared for combustion in the tissues. It is of great practical importance, therefore, that chemical methods should have been found which enable us to distinguish the characteristic properties of each of these types of fat. The chemical properties which have been used for this purpose are the iodine value and the percentage yield of fatty acid of the fat. The iodine value is a measure of the unsaturated fatty acids present and is highest in the tissue fat, lowest in the depot fat, and it usually occupies an intermediate but variable position in the case of liver fat. The percentage of fatty acid indicates the size of the molecule into which the fatty acid is built, being high in the case of the simple glycerides (neutral fat) and low in the case of the phospho-lipins (lecithin, etc.). This percentage is lowest in the tissue fat, highest in the depot fat, and like the I-value it usually occupies an intermediate but variable position in the fat of the liver. These chemical differences between the depot, the tissue and the liver fat have made it perfectly clear that the ordinary history of fat consists in its deposition in the depots and the subsequent withdrawal of this fat for combustion purposes in the tissues, an intermediate stage, however, being transportation to the liver, where it is prepared for tissue consumption. The nature of this preparation of fat which occurs in the liver has been very carefully and thoroughly studied by J. B. Leathes and his pupils. It is work the initial stages of which were completed several years ago, and to which contributions are every now and then being made.

Of the recent contributions those of Coope and Mottram deserve especial mention.[11, 12, 13] These authors have observed that infiltration of the liver with fat occurs during pregnancy and early lactation in laboratory animals, particularly in the rabbit. They point out that all evidence, so far, that fat may migrate from the depots to the liver has been furnished by observations on animals under abnormal conditions; for example, either in phosphorus or phlorhizin poisoning in laboratory animals, or as a result of disease in man, as in cyclical vomiting, diabetes, etc. The above authors found that the fatty acid deposited in the liver in late pregnancy gives an I-value which lies nearer to that of the mesenteric fatty acid than is the case in normal animals. Mottram concludes that "wherever . . . there is abundant fat metabolism, the liver is found to be infiltrated with fats, presumably to be handed on elsewhere when worked up."[13] It is interesting that the fetus is greedy of unsaturated fatty acids.

A practical clinical application of the above work is that fats will be more readily utilized by the body when they contain a high percentage of unsaturated fatty acids. It is probably for this reason that Norwegian cod-liver oil is of such undoubted nutritive value. It is much more so than Newfoundland cod-liver oil because in the preparation of this variety oxidation occurs, thus making it no longer unsaturated. Fish oils in general are more unsaturated than other animal oils, and are for this reason more nutritious.

The high percentage of unsaturated fatty acid in the liver as compared with that in the depots need not, however, *necessarily* indicate that the liver has produced the desaturation, for it might be that his viscus has high attractive power for unsaturated acids that is to say, that unsaturated acids circulating in the organism are especially appropriated by the liver cells. In order to test the hypothesis that the process of desaturation does really go on in the liver, Raper[10] after trying various methods studied the I-value of the volatile fatty acids of the liver after feeding or injecting animals with cocoanut oil. This oil is volatile in steam, the distillate being saturated, that is to say, having no iodine value. Although such a distillate of normal liver has a certain iodine value of its own, it was hoped that after feeding cocoanut oil a change might occur in the distillate from the liver, indicating that desaturation of the absorbed or injected fatty acids had occurred. It was found in some experiments that the volatile oils obtained from the liver, after giving the cocoanut oil, absorbed more iodine than those from a normal liver.

One interesting outcome of this research was the fact that in anesthetized animals cocoanut oil, placed in the intestine along with glycerine and bile salts, was absorbed to the extent of 30 per cent by the liver. When given in fine emulsion intravenously from 25 to 60 per cent of it was found in the liver. This retention of the oil by the liver may of course be due in part at least to the high and sudden concentration of the oil in the blood, and it may be partly due to the anesthetic.

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—*J. J. R. M.*

Intraspinal Use of Phenolsulphonphthalein

THE most convincing investigation of hydrocephalus is that of Dandy and Blackfan.[1] Their work has frequently been quoted as experimental corroboration of the rationale of intraspinous medication.[2]

The importance of their conclusions lies, first, in their demonstration of the feasibility of injecting phenolsulphonphthalein into the spinal canal. Long experience with this drug in functional kidney tests has proved it inert and capable of accurate quantitative detection. Secondly, they use pressure never over normal. Lastly, they were able to completely verify their animal experiments on human beings.

They injected 6 mg. of the drug in 1 c.c. of water—a neutral solution, of course. In normals, when injection was made into the cisterna magna, they could promptly recover it from the ventricles, and when injected into the ventricles, from the spinal subarachnoid. Without pressure they could inject the whole spinal and cerebral subarachnoid with insoluble granules. By experiments on normal human beings, they thus established three standards for phenolsulphonphthalein: (a) the time (1-3 minutes) of appearance of the drug in the spinal fluid after injection into the ventricles; (b) the time (10-12 minutes) and the rate (12-20 per cent in two hours) of excretion in the urine after ventricular injection; and (c) the time (6-8 minutes) and the rate (35-60 per cent) of excretion after subarachnoid injection.

In nine hydrocephalic children they were able to show that after spinal injection the drug appeared in the urine in normal time and amount, but that after ventricular injection it did not appear in the urine at all for the reason that it did not pass out into the subarachnoid space. Seven of these cases came to autopsy and in all the experimental findings were confirmed by demonstration of lesions mechanically obstructing the iter or foramina.

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[1]Dandy and Blackfan: Am. Jour. Dis. of Child., Dec., 1914
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Four other cases of hydrocephalus showed a normal passage of fluid from the ventricles to the subarachnoid, but here the absorption from the later space was delayed, averaging only 10 per cent in two hours. As the renal elimination was proved normal in all these, the assumption is justified that there is a type of hydrocephalus dependent solely on insufficient elimination from the subarachnoid. No autopsies were obtained in these cases.

In discussing the possible egress of spinal fluid through the pacchionian granulations, Dandy and Blackfan say—"Any evidence of the passage of fluid through the pacchionian granulations during life would be very difficult, if not impossible, to obtain. Consequently all proof is dependent on postmortem injections. In such a condition it is possible to force fluid through the pacchionian granulations with very high pressure." It seems strange that they should not have been familiar with the work of Cushing and his co-workers,[3] published only three months before their own. As a matter of fact, using living animals and only moderate pressures (180 mm. of water), Cushing did the very thing in question. True solutions—potassium ferrocyanide and iron-ammonium-citrate were precipitated after death by fixation in acid formalin and by the Prussian blue reaction were readily detected in the very act of passing through the walls of the pacchionian granulations into the sinuses.

As mentioned above, the work of Dandy and Blackfan has been quoted in favor of intraspinous therapy. If one were treating a purely luetic meningitis, one would be content with diffusion of his drug through the subarachnoid and their work substantiates the possibility of that. In endarteritis or general paralysis however the pericapillary and perineuronal spaces would have to be reached. Dandy and Blackfan did not secure such an injection. But in the work of Cushing the evidence wanted may be found, for, using pressures of 50 mm. of mercury, he secured a perivascular, perineuronal and, selectively, for some large cortical motor cells, an intracellular injection! The mere fact that phenolsulphonphthalein appears in the urine obviously supposes its passage through the blood vessels, but neither Dandy and Blackfan, nor Cushing and his co-workers believe in an absorption of spinal fluid by the cerebral end-vessels. Only in that way could the drug bathe the end-vessels and nerve cells in passing out into the circulation. To settle the question conclusively we must wait until someone has the good fortune to secure an autopsy not long after an intraspinous injection and demonstrates the presence of the drug in the structures we wish to reach. It is possible that the drug is absorbed from the subarachnoid without penetrating the parenchyma of the brain.

Other conclusions of Dandy and Blackfan are of minor interest confirming fairly established theories; i. e., experimental ligation of the vena Galeni magna produces hydrocephalus if it is made high enough to preclude collateral circulation; experimental hydrocephalus is less severe if the choroids are partly ligated before the iter is mechanically obstructed; venous congestion increases spinal fluid secretion; drugs and glandular extracts produce little effect; substances in solution in the blood and formed elements do not pass into the spinal fluid; in the dog, the fluid is restored every four or six hours; the lymphatics

[3]Cushing, Weed, and Wegefarth: Jour. Med. Research, Sept., 1914.

play a very minor role in the escape of fluid; there is no evidence of a current in the spinal fluid; the fluid passes out along the sheath of only the first, second and fifth cranial nerves; the probable cause of hydrocephalus after operations for meningocele is limitation of the excreting surface.

The harmlessness and utility of phenolsulphonphthalein injections in the human is the most valuable contribution of the above research. It would no doubt be of great value to other cases with symptoms of intracranial tension—luetic meningitis, delirium tremens, etc.

—*C. E. Kiely, per P. G. W.*

Cancer and X-Rays

IN 1912 Murphy[1] showed that an avian embryo has no defensive mechanism against the growth of tissues from a foreign species. He later showed[2] that tumor tissue from the rat could be transplanted in avian embryos for indefinite periods. Also he showed[3] that at about the time of hatching of an avian organism, a defensive mechanism developed which produced quick destruction of any foreign tissue. These observations were exceedingly interesting because they showed that heteroplastic grafting was possible providing the proper environment was furnished the graft. In these experiments the only morphologic facts observed were that in the young embryo there was no round cell infiltration about the foreign tissue, and that at about the time of hatching such an infiltration became noticeable. The only difference that could be discovered at the two periods was a difference involving the lymphocytes. With this in mind cultures of rat sarcoma in chicken plasma were prepared and to these were added bits of chicken tissues.[4] The sarcoma tissue grew well in plasma alone, and in cultures containing adult chicken connective tissue, kidney, and liver. When, however, chicken spleen was added to a culture, growth of the sarcoma tissue stopped. Bone marrow gave the same result. These experiments were repeated using chicken embryos in the shell, with the same results, except that bone marrow proved to be less effective than the spleen in inhibiting growth. Still later Murphy[5] showed that if an adult animal was deprived of a large amount of its lymphoid system by means of small repeated doses of x-ray, it no longer resisted the growth of heterologous tissue. The tissue cells of a foreign species will grow actively till such a time as the depleted system of the animal is well advanced in regeneration. This observation served to point emphatically to the role of the lymphoid tissue in tissue immunity. Murphy and Morton[6] thereupon studied the blood picture in mice and were able to observe that in naturally immune animals the mononuclear count rose immediately after inoculation with tumor material, while in susceptible mice this did not happen. They showed that resistance to transplanted tumor tissue is accompanied by a marked lymphocytosis. To test the suggestion that the lymphocytes were indeed the active agents in this immunity, they thereupon used the method of Murphy and Ellis[7] for destroying the major portion of the lymphoid system. They used the Coolidge tube, 10 milliamperes, 3 inch spark gap and an exposure of 1-2 minutes for several consecutive days, and

produced therewith a marked reduction of circulating lymphocytes. These x-rayed animals were then given an immunizing dose of blood, and were inoculated with tumor tissue, and compared with an immune series and controls. In the rayed series the incidence of growth was the same as in the controls, but the rate of growth was more rapid.

Murphy and Morton have also observed[8] that after depletion of the lymphoid system by x-ray, regeneration will commence after a time and progress actively to a point where there is actually an overproduction of lymphoid cells. They also noted that by using a very small dosage of rays they could produce stimulation of the lymphoid structure. This naturally suggested a study of the therapeutic effects of the x-rays, and it was made in such a manner as to indicate the differences between the effects of the rays upon the animal as a whole, upon the tumor in it, and upon the tumor outside the body. To produce the necessary conditions they took mice in which cancers were growing and from them they removed the tumors temporarily while they gave stimulating doses of x-rays to the hosts, using the Coolidge tube. Immediately after treatment the cancers were grafted back into their hosts. In 50 per cent of these animals there was a complete immunity against the grafts. As controls they used animals affected in exactly the same manner as in the former group, and these were treated exactly as were the others except that the raying was omitted. The cancers were removed and then grafted back again. In 28 of 29 animals so treated the grafts grew progressively. In a third group (10 animals) the tumors were removed, treated with rays in the same dosage used previously on the animals, and then grafted back. In 100 per cent of these the grafts took and grew.

In this series of researches, there seems to be good experimental evidence that immunity to tumors (some at least) in mice is closely associated with the activities of the lymphoid tissues of the animals; and that the x-ray, given in proper amounts, may stimulate these lymphoid tissues and so render the animal resistant to tumor growth, or, in heavy doses, may destroy, temporarily, the lymphoid tissues, and so decrease resistance. With the ever increasing use of x-rays (to say nothing of radium) in the treatment of tumors in human beings, it would be of value to study the effects of treatments upon the tumor itself and upon the blood picture of the patient. It seems that a possibility exists that a differential blood count may be of very distinct value in indicating the course the treatments should follow. It would be exceedingly interesting to know whether or not the lymphocyte count can be made to rise or fall by x-ray treatments, and whether or not these fluctuations are related in any way to the therapeutic effects upon the tumors.

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—*P. G. W.*

The Presence of Eosinophile Cells in Pleural Exudates

INTEREST in the cytology of exudates in the serous cavities in the past has centered chiefly in the lymphocytes, endothelial cells and neutrophilic leukocytes. Evidence is accumulating, however, which shows that an important role is played by the eosinophiles at times.

E. Rist and de Pfeffel[1] have found, in a study of the exudates which follow in the course of artificial pneumothorax, that eosinophiles and mast cells are often conspicuous. The cytologic formula varies, moreover, with the duration of the exudate.

In exudates of recent formation, the authors find that eosinophilic leukocytes predominate, though they constantly find some mast cells. At times, they have found that these two varieties of leukocytes alone are present in the fluid. But, if the exudate persists, lymphocytes begin to collect in the fluid, few at first, though later they may predominate. At the time when the fluid shows an excess of eosinophiles and mast cells, the blood may contain 5 to 6 per cent of the former and 2 per cent of the latter.

In cases of spontaneous pneumothorax, Rist and de Pfeffel have found fluids containing eosinophiles and mast cells without neutrophiles, though more often neutrophiles are greatly in the majority.

Occasionally, they have seen the eosinophile mast-cell picture in the earliest stages of tuberculous pleurisy; soon, however, the usual lymphocytosis replaces it.

In the pleural effusions complicating lobar pneumonia, eosinophiles have been conspicuous in eight cases, according to S. Bayne-Jones,[2] who reports a case. Mast cells were also present in his case. Among the other conditions with which pleural eosinophilia has been found, according to Bayne-Jones' review of the literature, are trauma, sepsis, typhoid fever, syphilis, polyarthritis, nephritis, pulmonary gangrene, hemorrhagic infarct of the lung, endothelioma, septic endocarditis, gonococcal sepsis, myocarditis, cardiac insufficiency, puerperal sepsis, neoplasm, influenza.

The list of conditions is chaotic and the underlying cause or causes of the accumulation of eosinophilic and basophilic leukocytes do not seem evident. This only indicates the need of more systematic study of the cytology of exudates. Schwarz[3] in 1914 was able to find but 68 cases in the literature, in which exudates showed an eosinophilia. Bayne-Jones, however, believes the phenomenon is of much more frequent occurrence than this would seem to indicate.

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—*R. S. M.*